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A BACTERIAL SURVEY OF SOME NEW ZEALAND FOREST LANDS, GRASSLANDS, AND PEATS

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(*Received 6 September 1960*)

Summary

Samples of forest litters, forest soils, grassland soils, and peats from throughout New Zealand and the adjacent islands were examined. A number of the samples were analysed chemically and the faunae of several litter samples were examined.

Differences in the size of the population and the incidence of bacteria, streptomyces, and sporogenous fungi are noted. Four distinct bacterial habitats are recognised: the growing leaf surface; the accumulated forest litter; mineral soil; and peat. The principal quantitative, taxonomic, and physiological features of the florae of these four habitats are described.

INTRODUCTION

Terrestrial life is dominated by the life cycle of plants, their growth and their decay. This life cycle defines the four habitats with which this paper is concerned: the growing leaf surface, the accumulated forest litter, the mineral soil, and peat. Within these four broad categories differences arise from dissimilarities in the character of the plant, the soil, and the climate; from the

intervention of secondary factors such as animal life; and, not least, through the passage of time.

Each of these four habitats possesses a distinctive bacterial flora. The leaf flora is readily recognisable by the predominance of pigmented forms. The soil flora has a high proportion of spore-forming bacteria (Stout 1958, 1960a, b). The flora of litter, particularly forest litter, derives partly, in the surface layers, from the leaf flora and partly, in the deeper layers, from the soil flora, but has, as well, a distinctive litter component in the more permanent litter layers. The flora of peat appears to be both physiologically and taxonomically distinct from the other three florae.

Taxonomic, physiological, and numerical differences in the various populations are very pronounced. The present survey was intended to give some indication of how far these differences could be attributed to the physical, chemical, and biological conditions of the environment; how closely they were related to the organic cycle; and how far the interrelation of the different groups of organisms affected the populations.

Materials and Methods

Over the last five years, as time and opportunity permitted, a diverse range of pasture leaves, mineral soil, litter, and peat samples were examined microbiologically. Counts were performed on the dilution plates and a number of representative strains were isolated for further study. Apart from this procedure, treatment of the sample depended upon the type of sample (that is, litter, soil, or peat), upon the desirability of testing various hypotheses, and upon the time which could be devoted to its examination. However, despite the diversity of treatment, the accumulated results do give some overall picture of the range of bacterial populations and the type of flora associated with the different soil niches.

The practice was to examine the samples as soon as possible—within a day or two—after their arrival at the laboratory. There was often a delay of a few days, however, between collection and arrival at the laboratory, and, in the case of samples from the outlying islands, this represented a delay of some weeks.

Samples were collected in plastic bags or in sterile 4 oz screw-capped jars.

Dilutions and Plating Media

Dilutions were prepared in 10-fold series, with duplicate plates poured from the 10^{-3} to 10^{-6} dilutions for soils, peats, and litters, and up to 10^{-9} for grasses.

The most generally employed plating medium was DTY agar (glucose 5 g, tryptone 5 g, and Difco yeast extract 4 g, agar 15 g, distilled water 1 litre). Difco Nutrient agar was used occasionally. Lactose agar, consisting of lactose 10 g, Difco yeast extract 1 g, agar 15 g, and distilled water 1 litre, was also used. Previously (Stout 1960b) it had been found that there tends to be a wide

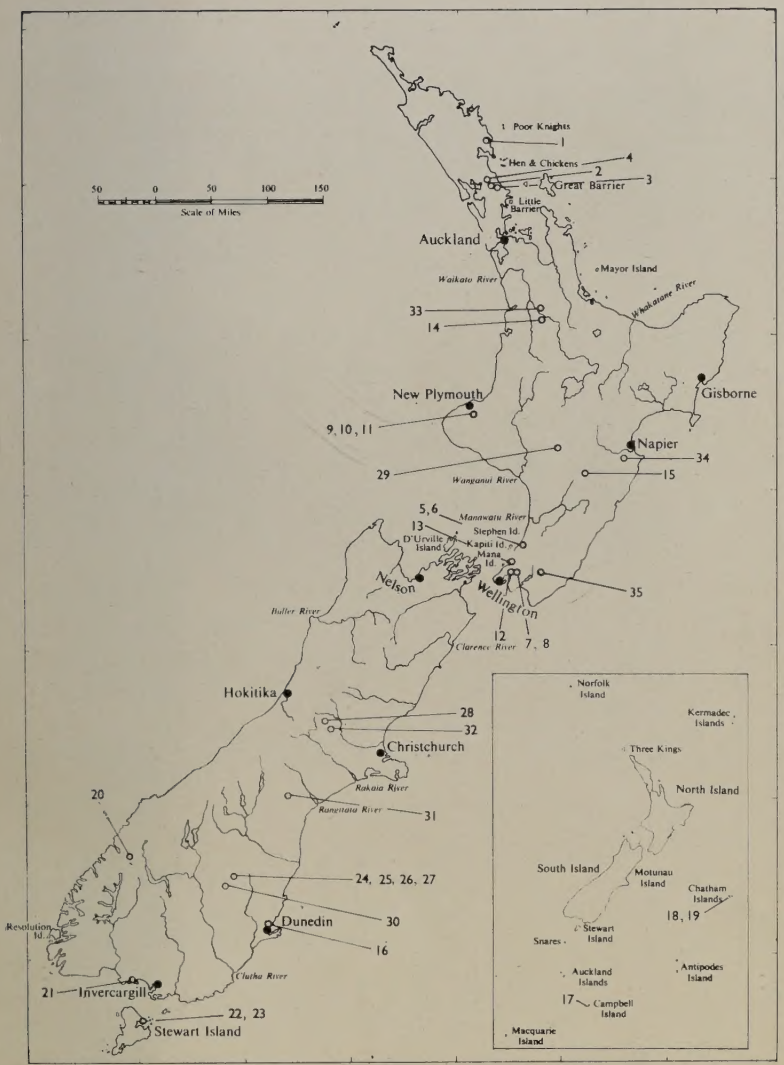

Fig. 1.—Locations of samples.

discrepancy between the counts on the glucose (DTY) agar plates and the lactose (Lactose-yeast extract) agar plates with samples of herbage (the G/L ratio), the glucose counts being higher than the lactose counts. This discrepancy disappears if the samples are stored some months before plating and if the plates are held several

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TABLE 1. DESCRIPTION AND CLASSIFICATION OF SAMPLES
(Each sample number refers to the locality indicated in Fig. 1)

Soil Classification	Soil Name	Samples	Vegetation	Locality
Zonal Soils				
Brown-grey earths	Linnburn soils	A. non-saline	sweet vernal (*Anthoxanthum odoratum*)	(24) Ida Valley, Central Otago
		A. solodised solonetz	*Puccinellia atropis*	(25)
		A. eroded solodised solonetz	none	(26)
		A. solonchak	none	(27)
Yellow-grey earth (related skeletal soil)	Tengawai steepland soil	A, B, C	tussock (*Danthonia, Festuca*)	(31) Orari hills, Geraldine
Yellow-brown earths weakly weathered, moderately to strongly leached	Kirkliston soils	A, B, C	tussock (*Danthonia, Festuca*)	(31) Orari hills, Geraldine
	Kaikoura steepland soil	A		
moderately weathered, moderately leached	Porirua sandy loam	A	browntop (*Agrostis tenuis*)	(13) Wellington
	Korokoro hill soils	A	pasture	(12) Wellington
	Taita hill soils	L	Monterey pine (*Pinus radiata*)	(7) Wellington
		F and H		
		A1	manuka scrub (*Leptospermum scoparium*)	(8) Wellington
		L		
		F		
		A1		
strongly weathered weakly podzolised	Rangiora clay	L	kauri (*Agatha australis*)	(1) North Auckland
		F		
		H		
		A1		
	Not named	H	kamahi (*Weinmannia racemosa*)	(22) Oban, Stewart Is.
	Not named	A1	pasture	(23) Oban, Stewart Is.
	Pukeraki fine sandy loam	L	matipo coastal forest (*Myrsine chathamica*)	(18) Chatham Is.

Intrazonal Soils				
Rendzina and associated yellow-brown earths	Arapohue clay	Litter (leaf and twigs) A1 A1	puriri (*Vitex lucens*) paspalum pasture (*Paspalum dilatatum*)	(3) North Auckland (4) North Auckland
	Maungaturoto clay complex	L F H A1	kauri (*Agathis australis*)	(2) North Auckland
Yellow-brown sand	Rapanui loamy sand	A1	karaka coastal forest (*Corynocarpus laevigata*)	(19) Chatham Is.
Yellow-brown loam	New Plymouth black loam	A1 (3 sites)	pasture	(9, 10, 11) New Plymouth
Organic soils	Moanatuatua peat	Brown sphagnum 0– 1 in. 11–13 in.	*Sphagnum*	(14) Hamilton
	Unnamed peat	Green sphagnum Brown sphagnum 4– 5 in. 6– 7 in. (2 samples)	*Sphagnum*	(15) Mokai Patea Ridge, Ruahine Ranges
	Kaherekoau peat	0– 1 in. 2– 3 in. (3 sites)	herbfield and *Dracophyllum* shrub	(16) Swampy hill, Dunedin
	Invercargill loamy peat	0– 3 in.	rush and manuka (*Juncus* sp. and *Leptospermum scoparium*)	(21) Colac Bay, Southland
	Unnamed peat	0– 1 in. 5– 7 in. 11–13 in. 17–19 in. 23–25 in. 26–27 in.	*Dracophyllum* and other shrubs	(17) S.E. Slope of Burnan hill, Campbell Is.
Azonal Soils				
Recent soils	Gollans silt loam	Litter (leaf and twigs) A1 Herbage A1	kohekohe (*Dysoxylum spectabile*) rye-grass and white clover pasture (*Lolium perenne* and *trifolium repens*)	(5) Wellington (6) Wellington
Subalpine regosol	Seaforth sand	A1	herbfield	(20) Lyttle's Flat, Upper Hollyford Valley, Southland

TABLE. 2. CHEMICAL ANALYSES

Sample	Horizon	Depth* in.	pH	Loss on Ignition %	Acid Insol. Ash %	Alk. of Ash me %
Rangiora soil under kauri (S.B. 7394 A, B, C, D)	L	(½)	5.5	93.3	1	105
	F	(1–1½)	6.0	85.8	8	111
	H	(4–6)	3.7	71.4	20	30
	A1	0–2	4.4			
Maungaturoto soil under kauri (S.B. 7395 A, B, C, D)	L	(2)	6.0	90.8	3	117
	F	(2)	7.4	71.5	19	143
	H	(½–1)	6.9	49.2	43	115
	A1	0–3	6.6			
Arapohue soil under puriri (S.B. 7393 A, B)	L	(2–3)	6.9	74.1	21	140
	A1	0–3	6.9			
Arapohue soil under pasture (S.B. 7392)	A1	0–3	5.9			
Gollans soil under kohekohe (S.B. 7396)	A1	0–3	6.2			
Gollans soil under pasture (S.B. 7396)	A1	0–3	5.8			
Taita soil under pine	L	(4)	4.96	94.3	2.30	56.7
	F&H	(2)	5.08	86.3	9.35	55.3
Taita soil under manuka†	L	(½–1)	5.4	86.7		49
	F	(1–2)	5.8	70.5		53
	A1	0–4	5.6			
New Plymouth roadside soil under pasture	A	0–2	5.6			
		2–5	5.5			
New Plymouth ploughed soil under pasture	A	0–2	5.9			
		2–5	5.7			
Korokoro soil under pasture	A	0–6	5.3			
Porirua soil under pasture	A	0–6	5.4			
Linnburn soil under pasture (S.B. 7389)	A	0–6	5.5			
Linnburn soil—solodized solonetz (S.B. 7390)	A	0–6	9.8			
Linnburn soil—eroded solodized solonetz (S.B. 7388)	A	0–6	10.2			
Linnburn soil—solonchak (S.B. 7391)	A	0–6	7.7			

* For the litter horizons the thickness, not the depth, is recorded.

† Taita soil under manuka: pH of actual samples; chemical analyses from Miller *et al.* (1955)

P Total mg %	Ca Total me %	Mg Total me %	K Total me %	Na Total me %	Organic C %	Total N %	C/N	C.E.C. me %	T.E.B. me %	Base Satn. %
38	83	20.8	0.8	1.0						
59	87	26.6	0.8	0.8						
46	12	17.9	0.8	1.0	7.0	0.30	23	32.3	2.92	9
36	99	15.4	0.7	1.5						
64	120	23.4	0.6	1.1						
60	105	26.2	0.6	0.8	8.1	0.48	17	43.6	21.3	49
67	125	19.0	0.8	0.8	5.6	0.53	11	51.6	28.0	54
					9.3	0.83	11	55.6	24.9	45
					4.9	0.46	11	28.4	12.1	43
					4.7	0.42	11	16.4	5.2	32
161	34.5	17.1	4.4	5.2	54 Est.	0.89	61			
165	36.0	19.0	3.0	5.2	49 Est.	1.37	36			
59	32.6	13.8	3.8							
69	36.0	14.5	4.8		6.3	0.46	14	21.5	9.3	43
246					13.2	1.1	12	37.5	16.9	45
250					11.0	0.93	12	33.7	9.9	30
214					13.4	1.0	13	41.4	23.1	55
192					11.0	0.95	11	36.7	17.2	47
					5.3	0.34	16	17.8	6.4	36
					5.2	0.37	14	19.7	6.8	36
					2.1	0.19	11	10.7	3.6	34
					1.5	0.15	10	8.1	6.5	80
					0.2	0.05	5	4.3	35.0	
					2.9	0.26	11	12.8	7.6	60

Sample	Soluble Salts (me %)	Moisture (%)
Linnburn soil under pasture	12.8	16.3
Linnburn soil—solodized solonetz	5.5	9.8
Linnburn soil—eroded solodized solonetz	6.5	12.1
Linnburn soil—solonchak	3.1	26.8

days. The inconsistency between the glucose and lactose counts, however, has not been observed with soil dilutions. For this reason samples were plated on the two media to test the incidence of this discrepancy and the consistency with which it was associated with the different kinds of samples.

It had also been found (Stout 1958) that the great majority of isolates from tussock grassland soils were able to use ammonium as the sole source of nitrogen but few were able to use nitrate nitrogen. Media were therefore prepared with a basal salt solution (Stout 1958), glucose 0.5% and agar 1.5%. The ammonium agar used the basal salt solution: $(NH_4)\ H_2PO_4$, 1.0 g; KCl, 0.2 g; $MgSO_4.7H_2O$, 0.2 g; distilled water, 1 litre (adjusted to pH 7.0 with N NaOH). The nitrate agar was similar except that KH_2PO_4 was substituted for the $(NH_4)\ H_2PO_4$, and $NaNO_3$ (0.1%) was added.

The plates were incubated at room temperature (ca. 16–18°c) or at 25°c. Sometimes a parallel series was incubated anaerobically in a McIntosh and Fildes jar.

Glucose-tryptone broth tubes with brom-thymol-blue as an indicator were sometimes inoculated directly from the serial dilutions.

Counting and Isolation

Counts were estimated as the number /g wet weight.

Particularly with the DTY agar plates colonial differentiation permitted an absolute count to be made not only of the colonies developing but also of the relative incidence of the principal taxa. Comparative counts were also made on the different series prepared with different media. An estimate of the mould and streptomycete numbers was also made.

In some cases, this completed the bacteriological analysis, but, more often, a number of representative colonies were isolated from the plates and further examined for their biochemical and physiological characteristics and for more exact taxonomic determination. The range of tests employed varied with the different isolates. The methods are described elsewhere (Stout 1958).

Description and Classification of Soils

A summary of the description and classification of soils appears in Table 1. The chemical analyses are given in Table 2. The locations of the samples are indicated in Fig. 1. The sample numbers in brackets (e.g. (1)) refer to this map. The faunae of the litter samples, extracted with a Berlese funnel, are given in Table 3.

Forest Soils and their Associated Grasslands

Kauri Profiles. Two profiles under kauri (*Agathis australis*) were examined. Both were in North Auckland. One, the Rangiora clay (1), derived from greywacke, was a weakly podzolised, strongly weathered, yellow brown earth and a typical kauri profile. The second,

TABLE 3. Faunae of the Litter Samples Extracted with the Berlese Funnel at 60°C

Forest Litter	Kauri (*Agathis australis*)			Puriri (*Vitex lucens*)
Soil Type	Maungaturoto clay complex			Arapohue clay
Horizons	L	F	H	L
Fauna				
Acari	150	100	4	155
Collembola	375	1,766	117	13
Coleoptera	6	6	1	0
Isopoda	0	3	2	0
Diplopoda	0	12	0	3
Araneae	2	0	0	6
Chelonethi	0	7	0	4
Lepidoptera (larvae)	7	0	0	22
Diptera (larvae)	4	6	1	1
Psocoptera	1	0	0	0
Oligochaeta	0	3	0	1
Mollusca	0	10	31	5
Volume of litter extracted (litres)	2	3	2	2
Mass of litter extracted (g)	370 (bark 34)	960	1,024	160

the Maungaturoto clay [2], was part of a complex derived from mixed mudstone and limestone which, under natural conditions, would support a broadleaf forest with some kauri. Both profiles had three clearly recognisable litter layers. The upper litter layer, L horizon, consisted of freshly fallen and undecomposed leaves, twigs, and catkins. The second layer, the F horizon, consisted of partly decomposed but still clearly recognisable elements of the kinds present in the L layer. The third layer, the H horizon, consisted of the plant debris broken down into relatively small fragments. Physically there was no apparent difference between the respective L horizons and F horizons of the two profiles, although the chemical analyses showed a higher proportion of calcium in the Maungaturoto profile which was also reflected in the ash alkalinity figures. There was also a smaller loss on ignition and a greater proportion of acid-insoluble ash in the F horizon of the Maungaturoto profile as compared with the Rangiora profile. The H horizon of the Rangiora profile was dark reddish-brown with few recognisable remains. The analyses revealed that it was extremely acid and this was associated with a low base status, particularly a very low calcium figure, and a low ash alkalinity. The loss on ignition was less, and acid-insoluble ash was greater, than in the L and F horizon and was roughly the same as the F layer of the Maungaturoto profile. The H horizon of the Maungaturoto profile was very dark brown and less red than the Rangiora H layer and contained many recognisable plant remains. Chemically, it was near neutral in reaction, with a relatively high base status and no marked reduction in the alkalinity

of the ash. However, the loss on ignition was low (49%) and the acid-insoluble ash high (43%), which sharply distinguished it from the other litter samples. The Rangiora topsoil was a grey silty clay loam stained by the overlying humus. It was extremely acid; very low in bases, with a very low base saturation; and relatively low in nitrogen, with a high C/N ratio. The Maungaturoto topsoil was a black silty clay loam. It was near neutral in reaction, high in bases with a medium base saturation, relatively higher in nitrogen, with a lower C/N ratio.

The Maungaturoto litter layers were put into a Berlese funnel. A large number of small snails were extracted, particularly from the H layer (Table 3). Collembola and mites (Acari) were present in all layers and were particularly numerous in the L and F layers. The most diverse fauna was present in the F layer. The density of the different litter layers varied considerably: the F layer was almost twice as dense as the L layer and the H layer almost three times as dense as the L layer.

The contrasting features of the two profiles are: first, from a pedological point of view—(a) the relative thicknesses of the litter layers, (b) the more complete physical degradation of the Rangiora H layer, and (c) differences in the colour and structure of the topsoil; and, secondly, from a chemical viewpoint—(a) the higher alkaline status of the Maungaturoto profile, reflecting the higher calcium content, (b) the greater proportion of non-organic matter in the Maungaturoto profile, particularly in the H layer or horizon, and (c) the marked distinction between the pH and base status of both the H layers and the topsoils.

Broadly, the differences may be attributed to the presence of limestone in the Maungaturoto profile and the associated mellowing of the humus and soil favouring soil organisms, which appear to be responsible for the greater mixing of organic and mineral matter, particularly in the H horizon, and this appears to be associated with a more complete and rapid mineralisation of the dead plant material.

Rendzina Profile. Two rendzina profiles, one under broadleaf forest and one under introduced pasture, were examined. Both were in North Auckland and were of the same soil type, Arapohue clay, derived from limestone. The forest sample[3] was taken from beside the trunk of a large, old puriri (*Vitex lucens*) and had a litter layer, some 2–3 in. deep, of leaves and twigs. The pasture of the second sample[4] was predominantly paspalum (*Paspalum dilatatum*), with about 30% white clover, crested dogstail, browntop, ratstail, and sweet vernal. This soil had been lightly topdressed with phosphate about every two to three years.

The puriri litter consisted of loosely assorted leaves and twigs; although some were partly decomposed, the different elements in the litter could easily be separated. Chemically, it was near neutral in reaction, with a high ash alkalinity due to a high proportion of calcium. The loss on ignition (74%) and the acid-insoluble ash (21%) indicated some mixing with the mineral soil.

The two topsoils were not very different in appearance, both being dark clay loams. Chemically, the pasture soil was moderately acid, with a lower base saturation that the forest soil, which was near neutral in reaction. The pasture soil, however, had a higher organic carbon content (9.3%) than the forest soil (5.6%), though the carbon/nitrogen ratio was the same in both soils.

The forest litter was put into a Berlese funnel and a variety of invertebrates were extracted (Table 3). Mites were the most numerous animals.

Gollans Silt Loam. A recent soil, Gollans silt loam, derived from colluvium, gleyed, under coastal forest[5] and introduced pasture[6] was examined. This soil is near Wellington. The forest samples were taken under coastal forest, predominantly kohekohe (*Dysoxylum spectabile*), and the pasture samples were taken in an adjacent area where the pasture was predominantly rye-grass (*Lolium* spp.) and white clover (*Trifolium repens*). The forest soil had a few scattered patches of thin litter, leaves and twigs, easily separable, though some of the leaves were partly decomposed.

The topsoils were generally similar in appearance, brown silty loams. Chemically, the pasture soil was more acid with a lower cation exchange capacity and lower total bases than the forest soil. They both had similar amounts of organic carbon but the forest soil appeared to be very slightly richer in nitrogen.

The forest litter, on examination, had a rich and diverse animal population.

Taita Hill Soil Profiles. Two profiles of Taita hill soil were examined at the Soil Bureau Experimental Station near Wellington. The Taita hill soil is a yellow-brown earth that is moderately weathered, moderately to strongly leached, and derived from greywacke, the present sites being largely derived from colluvium. One profile[7] was from under mature Monterey pine (*Pinus radiata*), about 30 years old and consisted of an L layer of about 4 in. of fine needles, and an F and H layer, about 2 in. deep, of partly decomposed litter mat overlying a dark greyish-brown heavy silt loam. The second profile[8] was under manuka scrub (*Leptospermum scoparium*) and similarly consisted of a layer of about 2 in. of undecomposed manuka litter, an underlying layer of about 2 in. of partly decomposed manuka litter, and the dark greyish-brown heavy silt loam topsoil.

Chemical analyses were made of the pine litter. Analyses of manuka litter and of the topsoil are given by Miller *et al.* (1955); these analyses are reproduced in Table 2. Chemically, there was surprisingly little difference between the pine and manuka litters. The pine litter was more acid (ca. pH 5.0) than the manuka (ca. pH 5.6), although the base figures were about the same. The concentration of phosphorus in the pine litter (161–165 mg %) was much higher than in the manuka litter (59–69 mg %). The principal differences in the litters are due to the character of the plant material itself: the resinous pine litter compacts in the lower litter to form a dark fibrous mat, while the manuka litter remains more discrete, with less distinction between the two layers and without the adhering resin.

Introduced Pasture Profiles. Three soils under introduced pasture were examined. One was the New Plymouth black loam, and samples were taken from three sites: (9, 10, 11) one, by the roadside, had been grazed; a second had been ploughed two-and-a-half years previously; and a third was a poor pasture of sandier texture. Detailed analyses of these sites are given by Saunders (1959).

The other two soils were the Korokora silt loam(12), described by di Menna (1957), and the Porirua sandy loam(13) (N.Z. Soil Bureau 1954).

South Island Profiles. Four South Island soils, under tussock, were examined. These were the Kaikoura steepland soil, the Kirkliston hill soil, the Kirkliston silt loam, and the Tengawai steepland soil.(31)

Stewart Island Profiles. Two Stewart Island soils: (22, 23) were studied. Although these soils, near Oban, have not been described, they appear to resemble the strongly leached podzolised yellow-brown earths in south-western Southland described by Wright (1951). The litter sample was a granular mor, pH 3.5. The topsoil sample under pasture was a red-brown silty loam with fine grass roots through it, pH 5.0.

Seaforth Sand. An examination was made of the subalpine regosol, Seaforth sand, obtained from under a herbfield at Lyttle's Flat in the Upper Hollyford Valley. This was a grey sand with few roots and leaves, pH 5.7(20).

Chatham Island Soils. These were the samples whose protozoan faunae and yeast florae are recorded by Wright (1959). One sample the Pukeraki soil, was of a dark brown raw humus, pH 5.2, taken under matipo coastal forest; and the second, the Rapanui soil, was of the mineral topsoil, a very dark grey friable loamy sand under karaka coastal forest, pH 5.5.(18, 19)

Organic Soils. The following peats were examined: Moanatuatua(14), a lowland sphagnum peat (Grange *et al.* 1939); a high-altitude (ca. 4,000 ft) sphagnum peat on the Mokai Patea Ridge(15) in the Ruahines (Moar 1956); the Koherekoau peat under herbfield and Dracophyllum shrubs on Swampy(16), at an altitude of ca. 2,000 ft (Cranwell 1953); the Invercargill peat from a drained swamp west of Colac Bay, Southland(21), where the swamp forest had apparently been burned and the vegetation was rush (*Juncus* sp.) and manuka (*Leptospermum scoparium*); a Campbell Island profile(17) under stunted shrubs (*Dracophyllum, Coprosma, Hymenaphyllum,* tussock, fern, and moss) overlying basaltic rock at ca. 500 ft.

These peats were all acid (Table 4), with a wide C/N ratio (ca. 50–60) and a very poor mineral status.

Saline Soils. These were collected in the Ida Valley, Central Otago, where a semi-arid climate (annual rainfall ca. 14 in.) results in the retention of salts in the upper horizons of the soil profile. The series of samples of Linnburn fine sandy loam collected included a solonchak(27), a solodised solonetz under *Puccinellia atropis,*(25) an eroded solodised solonetz,(26) and a sample under sweet vernal (*Anthoxanthum odoratum*).(24) The solonchak represents the gleyed

TABLE 4. pH of Peat Samples

Horizon	Moana-tuatua	Mokai Patea	Kaherekoau (Swampy)	Invercargill Peaty Loam (Colac Bay)	Campbell Island
Growing sphagnum		5.0			
Brown sphagnum	4.1–5.5	4.5			
0– 1 in.	4.1–4.5		3.7–4.2	4.3–4.5	5.1
2– 3 in.			3.6–3.9		
4– 5 in.		5.8			
6– 7 in.		5.5–5.7			4.3
11–13 in.	3.6–4.3				4.5
17–19 in.					4.3
23–25 in.					4.9
26–27 in. (rock bottom)					5.0

soils of the arid or semi-arid zones, the groundwater being salty. The present sample was taken from the bottom of a pond which holds water only part of the year. When the sample was collected the pond was dry and the surface was bare of vegetation. Nevertheless, organic matter does tend to accumulate at such a site, and this is indicated by the organic carbon figure. The solonetzic soils are the drained soils in which the salt tends to be retained because of low rainfall and high evaporation. This tendency is similarly shown by the apparently non-solonetzic soil under sweet vernal which, at the time of sampling, had a high salt content.

Results

Numbers of Bacteria

Tables 5, 6, and 7 summarise these results.

Forest litter counts varied from 10^4 to 10^7; forest soils from 10^5 to 10^6; pasture soils from 10^5 to 10^6; and peats from 10^4 to 10^6.

The Kauri Profiles: the Rangiora and Maungaturoto Soils. Generally, the counts were high, of the order of 10^6 to 10^7. The two exceptions were the L and H samples from the Rangiora profile, the former being appreciably higher, and the latter appreciably lower, than the other counts. Comparing the respective Rangiora and Maungaturoto horizons, the differences lay chiefly in respect to these two exceptional counts. The counts throughout the four Maungaturoto horizons showed little variation, while the Rangiora counts showed the peak in the L horizon and the depression in the H horizon. The GL ratio tended to be greater than 1 in the upper horizons and less than 1 in the lower horizons, though there was a marked discrepancy in the Rangiora topsoil. The ammonium plate counts, done with the Maungaturoto samples, indicated that most strains could use ammonium as a sole nitrogen source, but the nitrate plates, conversely, indicated that few strains could use nitrate.

Forest and Grasslands: the Arapohue and Gollans Soils. The forest profiles will be discussed first. The leaf counts of both the puriri and coastal forest litters were of the same order (ca. 10^6); the

TABLE 5. Bacterial Counts of Forests and Grasslands and the Regosol
(1,000s/g wet weight)

Profile and Plant Cover	Horizon	Bacteria				
		Glucose	Lactose	G/L	Nitrate	Ammonium
Rangiora soil (kauri)	L	54,000	22,000	2.5	n.d.	n.d.
	F	4,400	6,800	0.7		
	H	250	380	0.7		
		2,300	800	2.9		
Maungaturoto soil (kauri)	L	3,000	2,300	1.3	40	2,300
	F	1,200	360	3.3	60	5,000
	H	2,150	2,400	0.9	90	2,200
		500	1,060	0.5	120	240
Arapohue soil (puriri)	L (Leaf)	1,340	1,180	1.1	160	50
	L (Twigs)	57	70	0.8	5	4
	A	610	330	1.9	30	70
Arapohue soil (pasture)	A	390	260	1.5	20	20
Gollans soil (kohekohe)	L (Leaf)	1,400	800	1.8	n.d.	n.d.
	L (Twigs)	720	260	2.8		
	A	1,100	650	1.7		
Gollans soil (pasture)	(Grass)	61,000	11,000	5.5	n.d.	n.d.
	A	1,600	1,500	1.0		
Taita soil (pine)	L	<100	<100		n.d.	n.d.
	F & H	3,600	1,700	2.1		
	A	950	750	1.3		
Taita soil (manaka) 1.	L	60	20	3.0	n.d.	n.d.
	F	110	60	1.8		
	A	500	300	1.7		
2.	L	100	50	2.0		
	F	50	40	1.1		
	A	900	700	1.3		
3.	L	340	170	2.0		
	F	20	10	2.0		
	A	600	100	6.0		
Stewart Is. soil (kamahi)	H	365	70	5.2	n.d.	n.d.
Stewart Is. soil (pasture)	A	310	245	1.2	n.d.	n.d.
Subalpine regosol (herbfield)	A	188	n.d.		n.d.	n.d.

Glucose: glucose-tryptone-yeast extract agar.
Lactose: lactose-yeast extract agar.
Nitrate: glucose, basal salts (including nitrate as nitrogen source), agar.
Ammonium: glucose, basal salts (including ammonium as nitrogen source), agar.
n.d.: not done.

twig counts were appreciably lower in both cases, but much more so with the puriri twigs, which were larger and woodier; and the topsoil counts were lower than the leaf counts, although, again, the difference was more marked with the puriri than with the coastal forest. With the exception of the puriri twigs, the G/L ratio was greater than 1 and tended to be wider with the coastal forest counts. The nitrate and ammonium counts done on the puriri profile samples indicated that only about 10% of the strains were able to use nitrate or ammonium as the sole nitrogen source. The pasture sample comparable to the puriri topsoil showed a lower total count and a narrower G/L ratio. The pasture sample comparable to the coastal forest topsoil had a higher count but, again, a narrower G/L ratio (near unity). The grass count from this pasture sample was high, as usual, with a wide G/L ratio (over 5) (cf. Stout 1960b).

The Two Taita Profiles, under Pine and Manuka. The three series of manuka samples revealed considerable variation in the litter counts, although the topsoil counts were fairly stable. Of the litter counts, the upper dry litter tended to have a higher count than the lower moist litter, and the G/L ratio tended to narrow in the lower litter. The pine profile showed a topsoil count similar to that of the manuka topsoil, with an apparently similar G/L ratio. The pine needles had a relatively low count, with a G/L ratio near unity, while the matted lower litter had a relatively high count, with a G/L ratio similar to the manuka upper litter.

The Two Stewart Island Samples. The forest sample glucose (DTY) count was comparable to that of the acid H zone count of the Rangiora kauri litter, although the G/L ratio was markedly different. The pasture soil had a lower count than the other grassland soils but was still of the same order, with a narrow G/L ratio.

Organic Soils. In the Moanatuatua bog there was little difference between the counts of the different horizons; but the G/L ratio, greater than unity, tended to increase with depth. In the Mokai Patea samples, however, the G/L ratio decreased with increasing depth. In the Swampy bog, which, unlike the first two, was not a sphagnum profile, the total count was in two of the three series appreciably higher in the surface sample, and, in these two instances, the G/L ratio was unity at the surface and widened in the lower sample. The anaerobic glucose count was lower in the upper sample than in the lower sample of these two series.

The Southland peat at Colac Bay had counts of the same order as the other peat samples, but was unusual in having a proportionately higher count on the lactose plates.

The Campbell Island samples taken from a profile under stunted scrub showed a very high count, relatively, for the surface (0–1 in.) peat, a marked drop in numbers in the immediate sub-surface (5–13 in.) layers, and a further increase in the deepest layers, particularly in the peat overlying the basaltic rock bottom (26–27 in.).

Saline Soils. Only the eroded solodised solonetz showed a higher count on the salt agar plates than on the non-salt plates, and, in this

TABLE 6. Bacterial and Mould Counts and Incidence of Fermenters in Organic Soils
(1,000s/g wet weight)

Profile And Plant Cover	Horizon	Bacteria		
		Glucose	Lactose	G/L Ratio
Moanatuatua (sphagnum)				
a. Brown sphagnum		106	54	2
	0– 1 in.	340	260	1.3
	11–13 in.	168	80	2
b. Brown sphagnum		100	80	1.3
	0– 1 in.	160	71	2.2
	11–13 in.	180	64	3
c. Brown sphagnum		720	570	1.3
	0– 1 in.	122	57	2.1
	11–13 in.	166	48	3.5
Colac Bay (rush and manuka)		360	465	0.8

Mokai Patea (sphagnum)		Bacteria			Glucose Fermentation		
		Glucose	Lactose	G/L Ratio	A & G	A	Alk
Growing sphagnum		250	75	3.3	.025	1	100
Brown sphagnum		57	30	2	.025	100	
	4– 5 in.	170	135	1.2	1	100	
	6– 7 in.	260	200	1.3	10	100	
		54	47	1	1	10	100

Swampy (herbfield and *Dracophyllum* shrub)		Bacteria				Moulds
		Glucose	Lactose	G/L Ratio	Anaerobic (Glucose)	
	0– 1 in.	125	5	14	n.d.	95
	2– 3 in.	100	650	0.15	n.d.	4
	0– 1 in.	1,600	1,600	1	40	180
	2– 3 in.	800	90	9	480	6
	0– 1 in.	1,200	1,200	1	100	140
	2– 3 in.	720	430	1.7	140	7

Campbell Is. (*Dracophyllum* and other shrubs)		Bacteria		Glucose Fermentation*	
		Gelatin Nutrient Agar	Liquefaction %	A & G	A
	0– 1 in.	6,200	75	100	
	5– 7 in.	41	50	<1	<1
	11–13 in.	35	25	<1	<1
	17–19 in.	129	25	<1	1
	23–25 in.	110	100	1	
	26–27 in.	350	100	1	

* aerobic and anaerobic.
n.d.: not done.

Glucose: glucose-tryptone-yeast extract agar.

Lactose: lactose-yeast extract agar.

Glucose Fermentation: Production of Alkali (Alk.), Acid (A), or acid and gas (A & G) in glucose tryptone broth with bromthymol blue as indicator.

TABLE 7. Bacterial and Mould Counts of Saline Soils (1,000s/g wet weight)

Profile And Plant Cover	Horizon	Incubation Time	Bacteria			Moulds	
			Glucose	Glucose + 4% NaCl	Ratio	Glucose	Glucose + 4% NaCl
			pH 6.8	pH 8.0		pH 6.8	pH 8.0
Linnburn (pasture)	A	4 day	1,290	70	18		
		5 day	1,350	145	9	40	35
Linnburn solonetz (*Puccinellia*)	A	4 day	390	80	5		
		5 day	460	215	2	25	15
Linnburn eroded solonetz	A	4 day	14	79	0.2		
		5 day	30	75	0.4	none	none
Linnburn solonchak	A	4 day	670	120	5.5		
		5 day	4,700	450	10	60	5

Glucose: glucose-tryptone-yeast extract agar.
Glucose + 4% NaCl: glucose-tryptone-yeast extract agar + 4% NaCl, pH adjusted to 8.0.

case, the total counts were very low. The difference was small and could be attributable to either pH or salt effects. The delayed growth in the case of the other solonetzic soil hardly suggests that a halophilic flora is dominant in the soil, and this is similarly the case with the solonchak and the pasture soil. The delayed growth of the non-halophilic flora in the solonchak suggests that, despite the relatively high numbers present, the bacterial flora is not very active or, perhaps, is active only during short periods particularly favourable for bacterial growth. Both the pasture soil counts and the solonetzic counts imply a more stable population.

Physiological Character of the Populations

In a number of instances in Table 6 some indication of the saccharolytic activity of the population is given by the growth in glucose broth of inocula from the successive dilutions. In addition, over 200 strains isolated from the various samples were inoculated into glucose broth, with brom-thymol-blue as indicator. The results are summarised in Table 8.

These results revealed a difference in the forest soils between the manuka and kauri profiles, on the one hand, and the puriri and the kohekohe, on the other. The former included a relatively high proportion of aerogenic fermenters (18–26%), and a relatively low proportion of strains failing to turn the broth acid, turning it alkaline (7–17%) instead. The latter had a much smaller proportion of aerogenic fermenters (5–10%) and a higher proportion of strains turning the glucose broth alkaline (21–34%). Apart from this overall difference of character, the figures show that in the kauri

TABLE 8. GLUCOSE METABOLISM OF ISOLATES
(Reaction in glucose-tryptone broth with brom-thymol-blue as indicator)

Profile and Plant Cover	Horizon	No. of Isolates and %			
		A & G	A	n.c.	Alk.
Maungaturoto (kauri)	L		3		1
	F		2		
	H	6	5		3
	A1		3		
		6 (26%)	13 (57%)		4 (17%)
Arapohue (puriri)	L (Leaf)	1	6		1
	L (Twig)		6		
	A1		2		3
		1 (5%)	14 (74%)		4 (21%)
Arapohue (pasture)	A1	5 (63%)	3 (37%)		
Gollans (kohekohe)	L (Leaf)	6	11	1	4
	L (Twig)		13		10
	A1	1	14		10
		7 (10%)	38 (55%)	1 (1%)	24 (34%)
Gollans (pasture)	(Grass)		22 (88%)	1 (4%)	2 (8%)
	A1		9 (56%)	1 (6%)	6 (38%)
Taita (manuka)	L	1	17		
	F	8	10		1
	A1	1	14		3
		10 (18%)	41 (75%)		4 (7%)
Linnburn (pasture)	A	5 (36%)	7 (50%)		2 (14%)
Linnburn solonetz (*Puccinellia*)	A	1 (10%)	9 (90%)		
Linnburn eroded solonetz	A		2		
Linnburn solonchak	A	3 (16%)	15 (79%)	1 (5%)	
Moanatuatua (sphagnum)	(Peat)	1 (10%)	3 (30%)		6 (60%)
Mokai Patea (sphagnum)	(Peat)	2 (12%)	5 (29%)	1 (6%)	9 (53%)
Swampy (herbfield)	(Peat)		15 (39%)	3 (8%)	20 (53%)

A & G—acid and gas; A—acid; n.c.—no change; Alk.—alkaline.

and manuka profiles the greatest concentration of aerogenic fermenters was in the lower litter layers (the H layer) and that they were relatively few in the other litter layers and in the topsoil. The puriri and kohekohe samples showed that the aerogenic fermenters present tended to be associated with the decomposing leaves rather than with the twigs or the topsoil.

The physiological character of the population of the pasture top soil associated with the kohekohe forest soil showed a general similarity to that of the forest topsoil; but the pasture topsoil on limestone from North Auckland, the Arapohue soil, was remarkable in the dominance of aerogenic fermenters in the population.

The peats were distinguished physiologically by the very high proportion (53–60%) of strains failing to produce acid and turning the glucose tryptone broth alkaline. Aerogenic fermenters, while present, did not form an important part of the flora. In the case of the Mokai Patea bog the fermenters appeared to be more numerous in the deeper layers of the peat. In the Campbell Island series they were present in large numbers (10^5 g wet wt.) at the surface, were rare or absent in the sub-surface samples, and recurred in the deeper layers. Similar results were obtained in the Campbell Island series with broths incubated aerobically and anaerobically, which suggests that the flora consists predominantly of aerobes and faculative anaerobes, rather than of obligate anaerobes.

Taxonomic Character of the Populations

In the previous section, considerable attention was directed to the incidence of aerogenic fermenters. Generally, three taxa are involved: the first includes yellow to orange pigmented strains which, almost invariably, have been isolated from the leaves of pasture plants (Stout 1960b); the second includes the deep red pigmented strains (*Serratia*) which have been isolated from certain grassland soils (Stout 1958, 1960a); the third includes the non-pigmented strains, the great majority of which conform to *Aerobacter* (*Cloaca*) *cloacae* type. These last have been isolated from a wide range of habitats: pasture leaves, forest litter, peats, Antarctic moss, and a very wide range of soils. This taxon constitutes, therefore, one of the most widespread of soil organisms. Other aerogenic fermenters, such as *Escherichia* or *Bacillus polymyxa,* never appear to be present in great numbers. This is also doubtless true of *Clostridium* spp. A presumptive total estimate of the *Aerobacter* population can be fairly readily determined by the simple procedure of inoculating glucose broth tubes from the serial dilutions from which the agar plates are inoculated. For this reason the incidence of this group, both relatively and absolutely, provides a useful criterion of the character of the flora; and, because it is so widespread, questions of a fortuitous distribution do not arise.

Of the remaining taxa lacking this physiological distinction of aerogenic fermentation, those producing characteristic pigments are the most valuable for ecological purposes, since their incidence may be readily assessed from the dilution plates. These, again, fall into several groups.

First are the pigmented or pigment-producing pseudomonads. The *Ps. fluorescens* group appears to fall into two taxa: one, failing to produce a strong brown pigment, is represented in soils of the semi-arid region of Central Otago associated with brown grey earths high in bases and occasionally high in salts; the other, producing a brown pigment as well as the blue-green pigments, is widespread in grassland soils occurring in the grassland soil in Stewart Island, and, in the North and South Islands, in soils of the humid zones—the yellow-brown earths and the yellow-brown loams. *Ps. aeruginosa* has never been isolated from any soil, litter, or peat sample. *Ps. chlororaphis,* a very characteristic taxon, has been isolated only from a brown-grey earth soil (Stout 1958). Pseudomonads producing dark brown pigment have been isolated both from grassland soils and from forest litter, but different taxa appear to be involved in each case. Yellow-pigmented strains (*Xanthomonas*) have been isolated almost invariably from pasture leaves.

The second group of pigmented strains comprise the yellow, orange, and orange-red isolates which form the greater part of the leaf flora. The great majority of them are Gram-negative rods belonging to *Flavobacterium* spp. Some, however, are micrococci or sarcinas.

One group that does not fit easily into these categories is *Chromobacterium.* The distribution of this genus is perplexing. It occurs occasionally, though never in great numbers, in grassland soils and in forest litters, and has sometimes formed a very high proportion of a leaf flora. Although widespread, its distribution appears to be irregular and does not seem to be associated with any particular ecological situation. In this respect, it has been found that colonies of *Chromobacterium* occur in greater numbers on the lactose plates than on the glucose plates, and that, occasionally, these colonies are altogether absent from the latter when well represented on the lactose plates. This suggests that the apparent anomaly of their distribution may perhaps be a reflection of the uncertainty of pigment production, achromogenic strains being more widespread than chromogenic strains. Such a possibility underlines the taxonomic difficulties of bacterial ecology.

The spore-forming bacteria commonly comprise an important part of the soil flora. They are less common in forest litters and peats, and occur in only relatively small numbers on leaves. The most widespread and the most common species are the large-celled taxa, *Bacillus cereus, Bacillus mycoides,* and *Bacillus megaterium.* Other species are less widely distributed and occur in relatively small numbers, *B. polymyxa* being perhaps the most common.

Micrococci and sarcinas occur on leaves and in forest litter but are rare in soil and peat. *Nocardia* occurs in soil and forest litter, and, on occasions, has been isolated from leaves, but it is never a dominant part of the flora. Myxobacteria have been isolated from forest litter and from a grassland soil formerly under forest.

Besides these relatively well-characterised groups, there occur in all habitats a varying but important proportion of Gram-negative rods, characteristically of limited biochemical activity, which are commonly referred to as the *Achromobacter* group. This is plainly a very

heterogenous collection whose taxonomic relations are very imperfectly understood.

At times equalling the population of the other bacteria, and almost invariably present in soil, are the streptomyces. As the most characteristic producers of antibiotic substances, they are necessarily a very important element of the soil microflora. They are conspicuously absent from the leaf flora but they may occur in appreciable numbers in forest litter. Fungi form a very important part of the flora of forest litter, proportionately a less dominant element in soil, and are more characteristic of the flora of decaying leaves than of the growing herbage.

The Flora of the Kauri Samples. Flavobacteria appeared to be dominant in the L and F layers. They were less dominant in the H layer, and few were in the soil. Spore-forming bacteria—*B. cereus* in the H layer and *B. mycoides* in the topsoil—occurred in these lower layers. The topsoil flora was essentially non-pigmented, and non-pigmented taxa, probably pseudomonads, were common in all horizons. Streptomyces of the order of 10^5–10^5 were present in all horizons, and moulds were of the order of 10^3–10^4.

The flora of the Maungaturoto series was similar to the Rangiora series in the incidence of *Flavobacterium* in the L layer. However, *Flavobacterium* also occurred in the H layer, in which fermenters (*Aerobacter cloacae*) were dominant. Spore-forming bacteria (*B. cereus* and *B. mycoides*) were present in the H layer and the topsoil. Streptomyces of the order of 10^5 were also present in these two layers. Moulds were more common in the litter layers (10^4–10^5) than in the topsoil (ca. 5,000/g wet wt.).

The Flora of the Broadleaf Forest and Pasture Soil Samples. The flora of the puriri leaves consisted predominantly of *Flavobacterium* (75%). *Aerobacter* formed about 5% and spore-formers (*B. cereus* and *B. mycoides*) about 15–20%. Streptomyces were of the order of 30,000/g wet wt. and moulds of the order of 20,000. *Flavobacterium* was less dominant in the twigs (45%) but the spore-formers (*B. cereus* and, particularly, *B. mycoides*) comprised about 40% of the colonies. Non-pigmented Gram-negative bacteria appeared to be *Achromabacter*. Streptomyces and moulds were of the order of 10,000–20,000. The puriri topsoil had less than 5% *Flavobacterium* and a similar proportion of spore-forming bacteria. The majority of colonies were either pseudomonads or achromobacters. Streptomyces were of the order of 10^5–10^5. Moulds were of the order of 10,000 to 15,000/g wet wt. The flora of the Aropohue topsoil under pasture had less than 25% flavobacteria, about an equal proportion of spore-formers (*B. cereus* and *B. mycoides*), and the remainder chiefly pseudomonads and aerobacters. *Chromobacterium* was also present. Streptomyces and moulds were of the order of 20,000–40,000/g wet wt.

The leaf flora of the kohekohe samples had few flavobacteria and micrococci. Spore-formers, particularly *B. mycoides*, made up about 10% of the flora. The majority, however, were pseudomonads or aerobacters. Streptomyces were of the order of 10^5–10^5 and moulds

ca. 1,400,000/g wet wt., i.e., about as numerous as the bacteria. Flavobacteria and micrococci also formed a small proportion of the twig flora, which was not unlike the leaf flora, consisting principally of Gram-negative bacteria but with no aerobacters. A single strain of *Nocardia* was also isolated from the twigs. Streptomyces were of the order of 10^5–10^6 and moulds ca. 4,200,000/g wet wt., i.e., about half the bacterial count but still appreciably higher than the leaf mould count. The forest topsoil had less than 20% flavobacteria. No micrococci were isolated. The great majority of colonies were bacilli, particularly *B. cereus, B. mycoides,* and *B. megaterium*.. Streptomyces were ca. 70,000/g wet wt. and moulds ca. 25,000/g wet wt., i.e., far less proportionately and absolutely than in the twig and leaf florae. The grass flora was as usual quite distinct from the soil or litter florae and consisted predominantly of pigmented colonies, yellow, orange, orange-red, pink, and red, representing various types of flavobacteria and micrococci. These pigmented colonies formed at least 75% of the flora. Bacilli were present but streptomyces were absent. There were ca. 1,000,000 moulds. The pasture topsoil consisted, by contrast, of non-pigmented colonies, almost wholly bacilli, with *B. mycoides, B. cereus,* and *B. megaterium* dominant as in the forest topsoil. Moulds and streptomyces were of the order of 10^4.

The Flora of the Pine and Manuka Samples. The flora of the pine needles consisted predominantly of moulds; numbering about 20,000,000/g wet wt., these moulds far exceeded the bacterial population of less than 100,000/g. Of the bacteria present *Flavobacterium* formed the most conspicuous part. Flavobacteria were also present in the humus layer, forming about 10% of the population; *B. cereus* and *B. mycoides* formed about 20% of the population, *B. cereus* being dominant. Pseudomonads and achromobacters apparently comprised the remainder of the population. Moulds were of the order 40,000/g wet wt. No flavobacteria were present in the soil flora, which appeared to consist chiefly of pseudomonads. Streptomyces and moulds of the order of 10^3–10^4 were also present.

Flavobacteria formed up to 50% of the flora of the upper manuka litter layer. *Chromobacterium* was also present and *Aerobacter*. Moulds were of the order of 10^4. The flora of the lower litter was similar, except that *B. cereus, B. mycoides,* and streptomyces were of the order of 10^3. As in the upper litter, moulds were of the order of 10^4. The topsoil differed chiefly in the absence of *Flavobacterium*. *B. cereus* and *B. mycoides* were present, as in the lower litter, and so were streptomyces. Moulds were of the same order as in the litter layers.

The Flora of the Grassland Soils under Tussock and Introduced Pasture. These samples are of interest because of the distribution of *Ps. fluorescens,* which was present in the Porirua and Korokoro soils but in only one of the New Plymouth samples, viz. the roadside pasture which had received no phosphate top-dressing. It was absent from the experimental top-dressed plot and also from the poor sandy pasture. Besides *Ps. fluorescens* the first sample had *Chromobacterium, B. mycoides* and other non-pigmented pseudomonads. The experimental plot had *Chromobacterium, Flavobacterium,* and *B. mycoides,* as well

as non-pigmented pseudomonads. Much the same flora was also present in the sandy soil.

Ps. fluorescens was also the dominant bacterium in the Kaikoura, Kirkliston, and Tengawai soils.

The Flora of the Stewart Island and Southland Samples. The flora of the subapline regosol includes *Chromobacterium* (ca. 9,000/g wet wt.), *Flavobacterium,* and *Bacillus cereus.* The Stewart Island pasture also had *Chromobacterium* present in appreciable numbers (ca. 21,000/g wet wt.), as well as *B. cereus, B. mycoides,* and streptomyces. *Flavobacterium* and *Serratia* were present. The flora was, however, predominantly pseudomonads, including *Ps. fluorescens.* The forest mor flora consisted predominantly of *Aerobacter,* with flavobacteria, pseudomonads, streptomyces, and moulds also present.

The Flora of the Chatham Island Samples. The bacterial flora of the Chatham Island peat consisted of *Flavobacterium* and *Chromobacterium.* Fungi were present, but not streptomyces, *B. cereus,* and *B. mycoides.* The mineral soil from the Chatham Island had a flora consisting of pseudomonads and bacilli (*B. mycoides*), as well as streptomyces and fungi. Together they indicated forest ecology rather than a typical peat ecology.

The Flora of the Organic Soils. No detailed taxonomic work has as yet been done on a peat profile. It is clear, however, from the biochemical tests that the flora is distinct from the soil or litter flora. Apart from the surface layers, where representatives of the leaf flora may be present, it differs also from the leaf flora. *Aerobacter* appears to be one bacterium, however, that is common to peat and to the other florae. One third of the isolates from the Mokai Patea peat proved to be spore-forming bacilli. One feature of the peat flora is that, although moulds may be common on the surface, in the lower layers they are generally very few. The Colac Bay peat sample contained a high proportion of *Flavobacterium. Chromobacterium, B. cereus, B. mycoides,* and pseudomonads were also present. There were moulds but not streptomyces.

The Flora of the Saline Soils. The flora of the eroded solodized solonetz was very restricted, with few bacteria and no moulds. The solonetz under *Puccinellia* had a flora including flavobacteria, pseudomonads, aerobacters, and bacilli, as well as a relatively high mould population (ca. 14,000/g wet wt.). It was distinguished, however, by the predominance of an unidentified bacterium of characteristic primrose yellow colonial growth. The solonchak was remarkable in the richness of its flora and in the occurrence of typical grassland taxa. *Pseudomonas fluorescens* formed about 15% of the population and *Flavobacterium* about 5%. *Serratia* and *Aerobacter* were also present. The colonies of *Ps. fluorescens* had initially a gelatinous character, with the pigment centred in small discrete parts of the colony and not uniformly present throughout. There were no streptomyces, but moulds were present in the order of 5,000–6,000/g wet wt. The pasture soil consisted predominantly of Gram-negative bacteria, including *Aerobacter,* with a few bacilli and flavobacteria. Moulds were present of the order of 10^4.

Discussion

General Character of the Populations

In previous papers the identity and character of the florae of grassland soils, tussock, and pasture leaves were established (Stout 1958, 1960a, b). The data presented in the present paper suggests that forest litter and peat may also have distinctive florae. More specialised florae are found in less extensive habitats, such as solonetzic soils, the silt of Antarctica's dry valleys (Flint and Stout 1960), or the sulphurous crater of an active volcano (Hamilton and Baumgart 1959). While there is little doubt as to the autonomy of these bacterial populations, little is known of their establishment and maintenance in the constantly changing environment of the earth's surface.

The most intensive work so far completed in New Zealand dealt with the grassland soil flora (Stout 1958, 1960a; Thornton 1958, 1960; Vernon 1958). Broadly, this consists predominantly of non-sporing fungi, streptomycetes, aerobic spore-forming bacteria, and Gram-negative bacteria of which pseudomonads tend to form a major part. This flora may be infiltrated to some extent by the carotenoid pigmented leaf flora, especially flavobacteria, but such infiltration is generally confined to the topmost layer of soil. The bacterial flora of grassland soils may consist very largely of the spore-forming bacilli (particularly *B. cereus, B. mycoides,* and *B. megaterium*), non-pigmented pseudomonads, and, present in small numbers at least, the aerogenic fermenter *Aerobacter cloacae*. Soils possessing this flora appear to be more prone to infiltration by the leaf flora. Organically rich soils (such as the Arapohue soil with an organic carbon content of 9.3%) show a generally similar flora but the aerogenic fermenters tend to be dominant. In the older grassland soils, or in soils adjoining them, the pseudomonad flora is characteristically dominant and is commonly distinguished by the presence of the *Ps. fluorescens* group. These older grassland soils also appear to show an increasing proportion of aerogenic fermenters in the organically richer soils, and this tendency is also accompanied by an increase in the number of taxa of aerogenic fermenters, namely, *Aeromonas,* and, particularly, *Serratia,* as well as *Aerobaster*. In this respect, therefore, one can consider the *Ps. fluorescens-Serratia* flora as an edaphic climax analogous to those found in plant ecology. (The (*Bacillus*-dominated non-pigmented flora may be considered a seral community.) This grassland climax base been found in New Zealand in soils of widely different origin, under native and exotic grassland associations, over a wide range of latitude, and, with one notable exception (that of the solonchak), it has not been found elsewhere. The typical sites were the Tekoa soil near Bealey,[28] the Taupo soil near Waiouru[29] (Stout 1958, 1960a), and the Stewart Island soil. A very similar flora, with *Ps. fluorescens* but lacking *Serratia,* is more widespread, and was found in the Omarama soil near Alexandra[30] (Stout 1958), the Kaikoura soil near Geraldine [31], the Cass soil near Broken River[32] (Stout 1960a), the Porirua and Korokoro soils near Wellington, and the New Plymouth soil near New Plymouth. In this last soil, however, *Ps. fluorescens* was present at only one of the three sites sampled, although there appeared to be

little difference betwen the soils at the three sites. Further, a number of soils which might have been expected to harbour this flora lacked *Ps. fluorescens* and tended in many cases to have a *Bacillus*-dominated flora. These included the Horotiu soil near Hamilton[33] (Stout 1960b), the Okawa silt loam near Hastings[34], and the Kokotau soil near Masterton[35] (unpublished). These differences, not apparently attributable to climatic, soil, or vegetational factors, may be considered to reflect the role of time in securing the establishment of the climax flora.

As previously pointed out (Stout 1960a), typical soil taxa, such as *Ps. fluorescens* and *Serratia,* are not found in the airborne flora. Their mode of distribution may therefore be restricted to passage through soil and consequently be slow and vicarious, subject to geographical and pedalogical barriers. Under such circumstances, while soils bordering on a grassland area would be readily colonised, soils in forested areas may take a considerable period before they can be invaded by this flora. This would account for the vicarious distribution of the flora, particularly in the North Island which, 100 years ago, was extensively forested and has only since then been converted largely to exotic grassland. (Some indication of this lag is shown by the Taita soil near Wellington, which is generally similar to the Korokoro soil. The particular area sampled had been cleared and under pasture for a period of over five years before a single colony of *Ps. fluorescens* was recorded on the dilution plates.)

Distinct from the soil flora is the flora of the growing green plant leaves. This commonly consists of predominantly pigmented bacteria, the pigments being carotenoids which appear to have a protective role against the effects of light and, particularly, photo-oxidation (Stout 1960a, b). Taxa include the pseudomonad *Xanthomonas*, the peritrichous *Flavobacterium, Micrococcus,* and *Sarcina. Pseudomonas* and *Bacillus,* common soil forms, are very poorly represented in the leaf population. The physiological and biochemical characteristics of the leaf population vary from plant to plant and over the seasons, doubtless reflecting the role of plant nutrients and the interplay of climatic factors such as moisture, temperature, and sunlight. Aerogenic fermenters are generally few, especially on the more hardened foliage. They may occur in considerable numbers on succulent green pasture leaves in the spring, and, should favourable plant growth conditions continue, this flora may persist well through into the summer. Leaf populations may reach very high figures, representing the highest non-parasitic bacterial populations in nature. Consequently, infiltration of the soil or of litter must be taking place all the time. Such infiltration can be recognised readily by the incidence of pigmented forms in the population. It does not persist extensively, however, either in time or space.

In pasture, dead leaves may form a transient litter of decaying vegetation. In forest this trend is accentuated to give a wide range in depth and stability of the litter of the forest floor. In peat it reaches its fullest expression with the persistent accumulation of undecomposed or incompletely decomposed plant remains. As is to be expected, the population of this litter tends to bear the impress of the original living

leaf flora. In the case of the litter—or hay—of pasture the population is very similar to the leaf flora, although the pigmented taxa are less dominant and there is a higher incidence of soil forms such as *Pseudomonas* and *Bacillus*. Sporogenous moulds are also very common and characteristically form the dominant element of the litter flora (Stout 1960b). The rapidly decomposing litter of the mull-forming trees, such as the puriri and the kohekohe, resembled the grass litter in possessing a flora related to both the leaf flora and the soil flora and without any distinctive litter elements, though the population differed between dead leaf and twig within the litter complex. In the deeper litters, such as the manuka and the kauri litters, a similar pattern obtained except that the leaf flora tended to predominate in the upper litter layers, while soil taxa, such as *Bacillus* and streptomyces, tended to predominate in the lower layers. The pine litter differed sharply from the other litters in the overwhelming predominance of moulds, an indication of the decisive role of plant material upon the litter flora. The differences between the two kauri litters appeared to lie chiefly in the size of the population, rather than in its taxonomic composition. This was particularly evident in the respective H horizons, where the differences between the two were most sharply defined. In the more stable litters, such as the manuka and kauri litters, there was a tendency for aerogenic fermenters (*Aerobacter cloacae*) to form a major part of the population in the well-decomposed layers, i.e., the H horizon of the Maungaturoto kauri profile and the F layer of the manuka profile. Aerogenic fermenters also appeared to predominate in the well-humified kamahi litter from Stewart Island.

The flora of the forest soil, as distinct from the forest litter, showed close affinities with the flora of the grassland soil. Sporogenous moulds tended to be less dominant, and streptomyces and *Bacillus* spp. formed a considerable part of the population. The more selective taxa of the grassland soils, however, were absent, e.g., *Ps. fluorescens* and *Serratia*.

The flora of organic soils differed quite sharply from the forest litter flora. Except for the surface of the bog where moulds and perhaps some pigmented bacteria were sometimes present, the peat flora was distinguished by the relative absence of moulds, streptomyces, and the pseudomonads characteristic of forest litter or grassland soil. Aerobic spore-forming bacteria were generally less common than in soil. The aerogenic fermenter (*Aerobacter cloacae*) was sometimes present. Most characteristic of the peat flora was its physiological inactivity and the tendency towards increasing anaerobiosis with increasing depth, e.g., Swampy. Detailed taxonomic work remains to be done on this flora.

Relation to the Chemical Analyses and the Pedology of the Soils

Three criteria may be used to compare different bacterial florae: the taxonomic distinction between Gram-positive and Gram-negative; the physiological distinction between fermenters and non-fermenters; and the cultural distinction between florae with carotenoid pigments and those without.

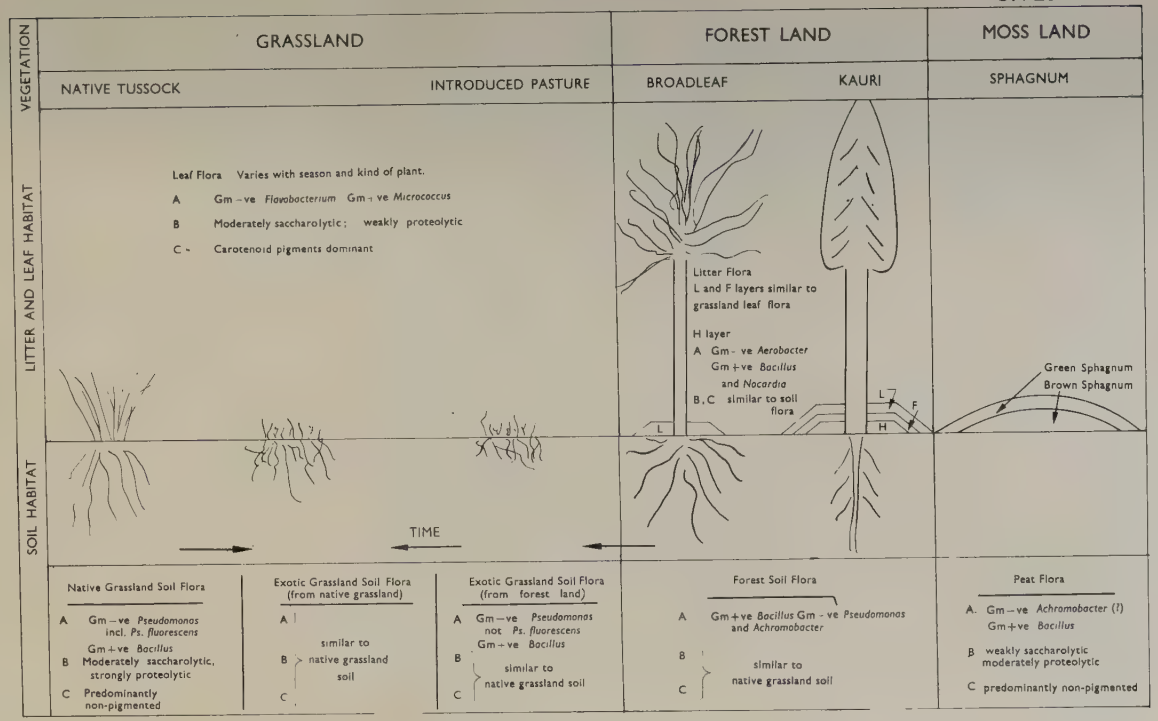

Fig. 2.—Tentative generalised diagrammatic representation of the principal habitats and the characteristics of their associated bacterial florae: (A) taxonomic character, the dominant group being shown first; (B) physiological character; (C) cultural character.

The florae of both mineral and organic soils could be distinguished from the flora of the growing leaf by: (a) a greater proportion of Gram-positive (especially spore-forming) bacteria; (b) greater proteolytic activity; and (c) the very small proportion of the carotenoid-pigmented bacteria which are dominant on the leaves. In forest litter the L and F layers tended to resemble more nearly the leaf flora and the H layer more nearly the soil layer.

These overall relations are illustrated in Fig. 2 in a simplified and diagrammatic form.

Within these broad limits differences may be correlated with differences in the organic carbon content or with exceptional soil properties such as high salinity. The dominance of fermenting bacteria in the H layer correlates with the accepted distinction of mull and mor. There appears to be less significance in the figures provided by chemical analysis, apart from organic carbon. pH and base status appeared to be of small significance within the limits of the present sampling, except in cases of extreme acidity such as the Rangiora H horizon. There appeared to be no particular significance in the concentration of the different bases nor in the phosphate figures.

There are not always exactly similar bacterial florae in samples from different sites of the same soil type, e.g., the occurrence of *Ps. fluorescens* at only one of the three samples of New Plymouth black loam.

The character and history of the vegetation, both at the site of sampling and within the adjacent area, appear to be of decisive significance in determining the taxonomic character of the flora, although its physiological character is more immediately the outcome of the plant and soil characters. The changes in the flora with changing conditions, and the development of what may be considered a climax population, appear to be very slow processes conditioned partly by the slow spread of soil organisms and perhaps partly by the persistence of adverse conditions.

Conclusions

(1) Four distinct florae are recognised as being associated with the four principal phases of the organic cycle, viz.: (a) leaves, (b) litter, (c) mineral soil, and (d) peat.

(2) The leaf flora is pigmented and is capable of being aerially distributed. Populations fluctuate greatly with seasons and vary with the type of plant. The G/L ratio tends to be much greater than unity.

(3) The litter flora is stratified; the upper layers are largely similar to the leaf flora and the lower layers are largely similar to the soil flora. The distinctive litter flora is associated with deep litters and is characterised by a high proportion of aerogenic fermenters, particularly in the well-humified layers. The G/L ratio tends to be greater than unity, especially in the upper litter layers.

(4) The mineral soil flora ranges from *Bacillus*-dominant to *Pseudomonas*-dominant populations; the first type of population is characteristic of most forest soils and of some grassland soils; the second type is characteristic of long-established grassland soils. The increasing proportion of Gram-negative bacteria and aerogenic fermenters is associated with an increasingly favourable environment. This sequence forms, therefore, an edaphic climax distinct from, but analogous to, the sequence of soil—rhizosphere—rhizoplane populations which is more strictly a plant climax. The G/L ratio tends to be greater than unity for forest topsoils but near unity for grassland topsoils.

(5) The peat flora is physiologically less active than the other florae and is less diverse. There may be increasing anaerobiosis with increasing depth. The G/L ratio tends to be greater than unity and may increase or decrease with increasing depth.

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SOME PRELIMINARY OBSERVATIONS ON FACTORS AFFECTING THE TEMPERATURE AT THE SKIN SURFACE OF THE NEW ZEALAND ROMNEY SHEEP

II. THE EFFECTS OF SYMPATHECTOMY AND OF VASODILATOR OR VASOCONSTRICTOR DRUGS

By F. Cockrem and G. A. Wickham,* Massey Agricultural College, Palmerston North

(*Received 31 August 1960*)

Summary

The effects of unilateral cervical sympathectomy and of various vasodilator and vasoconstrictor drugs on the skin temperature of the face of the Romney sheep are described. The results are discussed in relation to the use of such techniques in examining the relations of blood supply and wool growth. It was concluded that sympathectomy was the best experimental technique for increasing cutaneous blood supply over a long period without additional effects which might confound any experimental results.

Introduction

Methods of measurement of skin temperature were discussed in a previous paper in this series (Cockrem and Wickham 1960). The present paper describes some trials of various treatments which might be expected to affect cutaneous blood supply and hence skin temperature. These treatments might be of use in an experimental approach to the study of correlated wool growth effects. The possibilities of such an approach have been demonstrated by the action of a sympathomimetic agent on hair growth in mice (Cockrem 1959).

Methods

Skin temperatures were measured by the methods described previously (Cockrem and Wickham 1960). The four Romney ewes used were the same as those employed for these measurement trials; three of them (Nos. 7, 18, and 21) had had the right inferior cervical sympathetic ganglion and a portion of the cervical sympathetic nerve removed 10 months previously.

Treatments used were:

(i) Intra-muscular injections of Vasoxine [β hydroxy β (2,5 dimethoxyphenyl) iso-propylamine], 20 mg/ml.

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Vasoxine is a sympathomimetic pressor agent with a primary effect of increasing peripheral resistance. It differs from adrenaline in that it causes peripheral vasoconstriction without causing cardiac stimulation. It is also longer acting (Burroughs Wellcome, pers. comm.).

(ii) Subcutaneous injection of adrenaline hydrochloride in physiological saline.

The effects of adrenaline on peripheral circulation would be expected to be similar to those of Vasoxine but of a more transient nature. However, under some conditions, vasodilation, and not vasoconstriction, can occur. Adrenaline also has physiological effects besides those on the blood vessels (Houssay *et al.* 1951).

(iii) Ronicol (β pyridyl carbinol), 100 mg/2ml. Ronicol is a vasodilator substance with an action somewhat similar to that of nicotinic acid but with more prolonged effects. Wertheimer *et al.* (1955) found that Ronicol increased skin temperature in the human by 3.2°F in the blush area (head and cheek) but had no effect on the glamus area (toe). Kelvin (1954) has suggested that the increase in blood supply resulting from use of this drug in two bald patients led to a renewal of hair growth, but this effect of the drug has not been substantiated (B. J. Will, pers. comm.).

(iv) Presidal [N^1–(5 cyano 5–5 diphenylpentyl)–N^1 : N^1 : N^2 trimethylethylene–1 ammonium–2 morpholinium sulphate]. Presidal, a sympathetic- and parasympathetic-blocking agent (Adamson *et al.* 1956), was of interest in that some of the effects might be expected to be similar to those obtained by sympathectomy. However, Presidal would block the entire system, and the more extensive vasodilation resulting might prevent any marked increase in blood supply in any one region. However, the response to Vasoxine and adrenaline would be expected to be similar on the control and sympathectomised sides of the nose if the control side was "chemically sympathectomised."

(v) Reserpine (Serpasil injection). The effects of reserpine on the peripheral sympathetic system have been discussed by Muscholl and Vogt (1958). The effect might be similar to that of complete sympathectomy, but there are also other effects, in particular on the hypothalamus, which lead to a tranquilising action (Shore, Silver, and Brodie 1955).

Preliminary trials indicated that a repeated dose rate of 7.5 mg was liable to kill the sheep. The dose rate adopted was 2.5 mg. repeated after an interval of 48 hours. This led to a moderate degree of tranquilising for two–three days after the second dose. As the solvent in the Serpasil preparation has a slight transitory effect on blood pressure (McQueen, Doyle, and Smirk 1955), control animals were injected with the solvent only.

(vi) Ronicol compositum (Ronicol 100 mg, acetycholine 50 mg, pyridostigmine bromide 1 mg in a solvent of the polyethlene glycol ether of tetrahydrofurfuryl alcohol). Intramuscular injection.

The acetylcholine and the pyridostigmine bromide, which is an anticholinesterase, would be expected to enhance the action of Ronicol

by stimulation of parasympathetic vasodilator fibres. There would be additional effects on the remainder of the parasympathetic system and cholinergic fibres.

Results

Skin temperatures on the sympathectomised and control sides of the nose are presented before and after shearing, which was used as an additional treatment. Results for other body positions are given where they are relevant; but, in general, no major effects were found for the main body areas.

As no control of ambient temperature was possible in the shed available, results have been interpreted relative to this factor and to

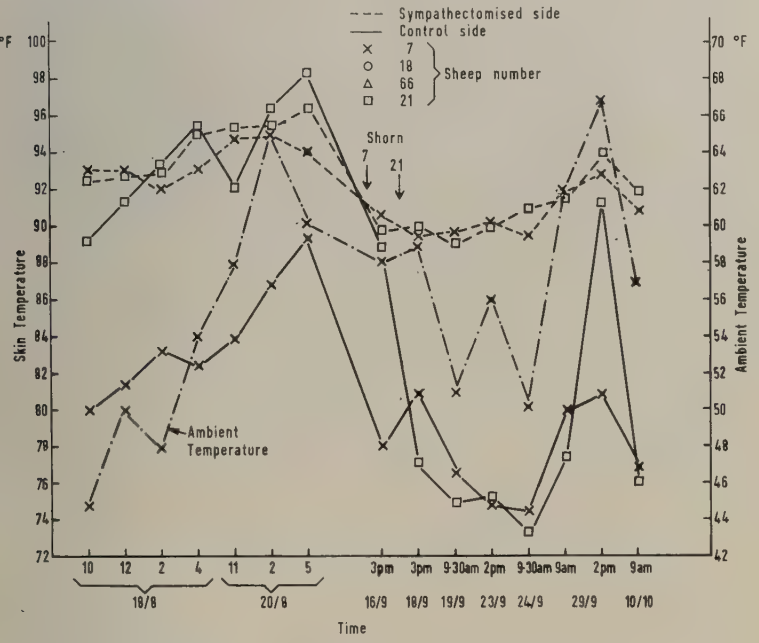

Fig. 1.—The effects of sympathectomy on skin temperature on the side of the nose before and after shearing. (Sheep Nos. 7 and 21.)

KEY FOR ALL FIGURES

Where the sheep are included the following key applies to all figures:

Sympathectomised side—broken line.
Control side—solid line.
Sheep No. 7—lines with crosses (except ambient temperature in Figs. 1 and 10).
Sheep No. 21—lines with squares.
Sheep No. 18—lines with circles.
Sheep No. 66—line with triangles.

the diurnal trend. Minor effects were not, therefore, likely to be detected.

The possible effects of injection itself were tested by measuring skin temperatures immediately before, and immediately after, the prick of a needle to inject saline. No response in skin temperature was found. However, sheep No. 18, whether injected or not, frequently showed a considerable drop (up to 9°F) over a period of 5–10 minutes when repeated readings were made on the control side of the nose.

The Effect of Sympathectomy

Skin temperatures on the sympathectomised and control sides of the nose for two of the sheep are shown in Fig. 1. The third animal had readings very similar to those of No. 21, and these have been omitted in the diagram for the sake of clarity.

The control side followed the ambient temperature, and its skin temperature was considerably reduced after the animals were shorn. The sympathectomy reduced the response both to ambient temperature and to shearing. For sheep No. 7 a considerable difference in skin temperature between the sides could be seen under all conditions, and in this animal the effect of sympathectomy could always be detected. For the other two, however, differences in favour of the sympathectomised side could only be found before midday or after they were shorn. It was clear that a marked effect of sympathectomy still existed 10 months after the operation.

Vasoxine

The results of the first trial are shown in Fig. 2. No effect of the initial injection of saline on the skin temperature of the nose was found and the control animal (No. 21) showed the normal diurnal trend. After injection of 4 ml Vasoxine, sheep No. 66 showed a

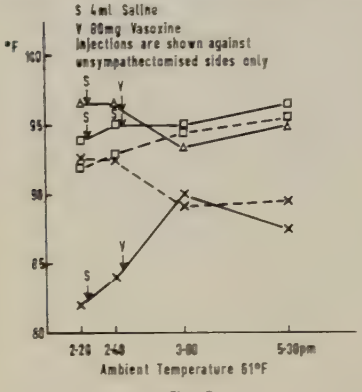

Fig. 2

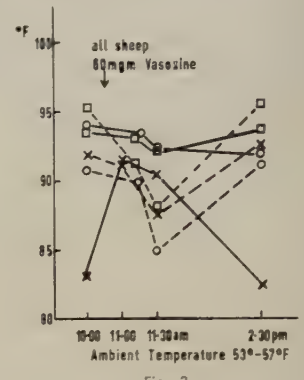

Fig. 3

Fig. 2.—The effect of 4 ml Vasoxine injection on skin temperature on the side of the nose. (Sheep Nos. 7, 21, and 66.)

Fig. 3.—The effect of 3 ml Vasoxine injection on skin temperature on the side of the nose. (Sheep Nos. 7, 18, and 21.)

slight depression of skin temperature for two to three hours. This was assumed to be a real effect as it was opposite to the normal diurnal trend.

For the same dose of Vasoxine, sheep No. 7 showed a marked reduction in temperature on the sympathectomised side but a rise on the control side. A small drop on the control side and a larger drop on the sympathectomised side would not be unexpected as this operation increases the sensitivity of the vessels to vasoconstrictor substances. This, in fact, occurred with sheep Nos. 18 and 31 when the trial was repeated with 3 ml Vasoxine (Fig. 3). Sheep No. 7 again showed a rise in temperature on the control side. Depressions of the order of 2°F were also observed on the poll and side positions. In all cases the effects lasted from two to three hours.

A third trial with a lower dose rate and with the measurement of skin temperature at shorter intervals is shown in Fig 4 for sheep 7. There was an immediate drop in temperature on the control side, followed by the rise previously observed. The response of the sympathectomised side appeared to be related to the dose rate.

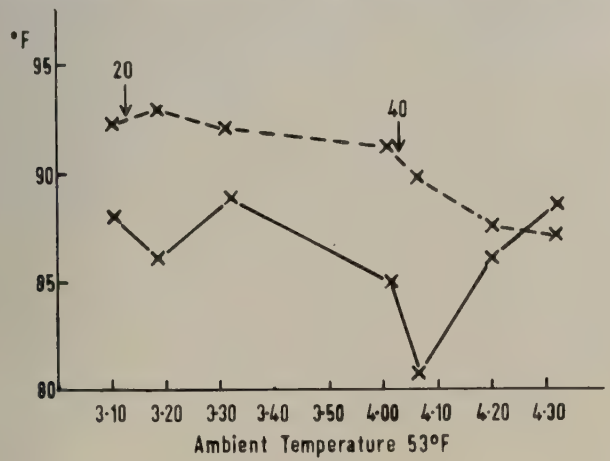

Fig. 4.—The immediate effects of Vasoxine injection (mg) on skin temperature on the side of the nose for sheep No. 7.

Adrenaline

Results for sheep No. 18 were not reliable, as the repeated readings over a short period only, caused a drop in temperature. Results for sheep No. 7 are shown in Fig. 5. Sheep No. 21 showed a slight reduction on the sympathectomised side only. Sheep No. 66 showed a slight transient drop in temperature in response to 0.2 μg. Sheep No. 7 showed little effect on the sympathectomised side for 0.1 μg adrenaline but a slight drop in skin temperature occurred with 0.2 μg. The control side, however, showed a slight drop followed by a marked

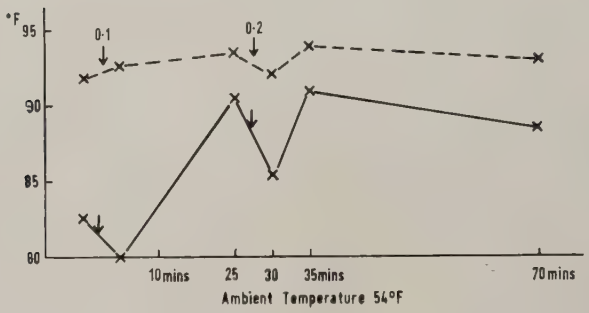

Fig. 5.—The effect of adrenaline injection (μg) on skin temperature on the side of the nose for sheep No. 7.

rise after 0.1 μg and a marked drop with no further rise after 0.2 μg. Thus 0.1 μg had an effect somewhat similar to that obtained with Vasoxine.

Ronicol and Ronicol Compositum

Before Shearing

Fig. 6 shows the effect of Ronicol injection (2 ml) on the three sympathectomised sheep. No control animals were used for this trial as the over-riding consideration for control was the individual behaviour already determined.

The sympathectomised sides showed no skin temperature changes of note. For the control sides sheep No. 21 showed no change and sheep No. 18 showed a small immediate rise which could have been

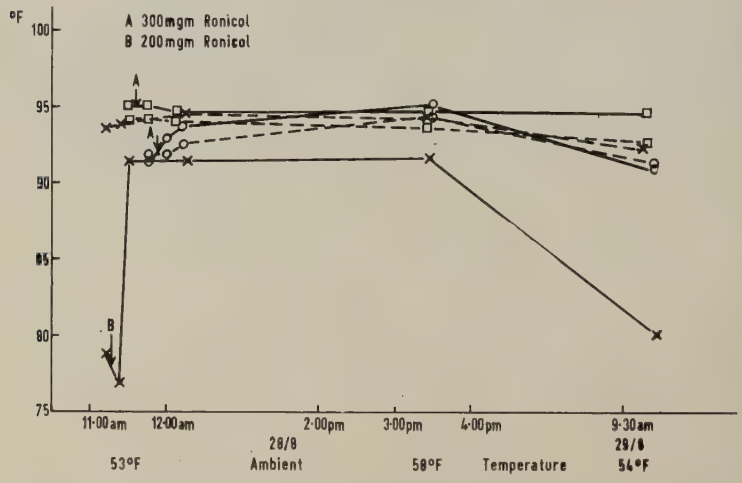

Fig. 6.—The effect of Ronicol injection on skin temperature on the side of the nose. (Sheep Nos. 7, 18, and 21.)

part of the normal daily trend. However, after a slight initial drop, sheep No. 7 showed a marked rise which was maintained throughout the day, but dropped to within the normal skin temperature range by the following morning.

It appears that a response to Ronicol may only result if the initial skin temperature is low, but clearly a larger number of sheep would be required to test a variable response of this nature. The temperature changes observed in other body positions could be interpreted as being the normal daily trend.

After Shearing

Ronicol Compositum. Fig. 7 shows the effects of Ronicol compositum on the face skin temperature. No effects were observed on skin temperature in the main body areas. An effect of the acetylcholine and anticholinesterase was seen in the stimulation of tears. A short-term increase in the temperature of the side of the nose was found for sheep No. 66 and on the control side of the nose of sheep No. 18.

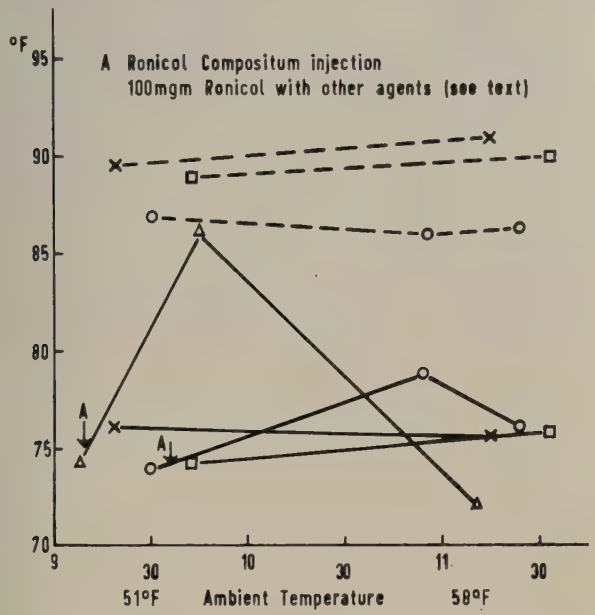

Fig. 7.—The effects of Ronicol compositum on the skin temperature of the side of the nose after shearing. (Sheep Nos. 7, 18, 21, and 66.)

Ronicol. After a recovery period of four days a further trial was made with Ronicol alone (two sheep 200 mg each, one sheep 400 mg, one sheep saline). No effect was found in any position. This contrasts with the preshearing and "compositum" results, and suggests

that the small increase obtained with the compositum was associated with acetylcholine.

Presidal

Before Shearing. The four sheep were injected with 25 mg Presidal after an initial skin temperature reading in the morning. Temperatures were again taken when the dilation of the pupil of the eye indicated that the drug was acting. After a further reading two hours later injections of adrenaline (0.1 μg) and then Vasoxine (3 ml) were made. For clarity sheep No. 18 is omitted from Fig. 8, in which the results are shown. Its response was similar to, but less marked than, that of Sheep No. 7. The Presidal did not prevent the drop in temperature shown by sheep No. 18 on the control side when successive readings were taken. Sheep No. 21 was not injected with Vasoxine.

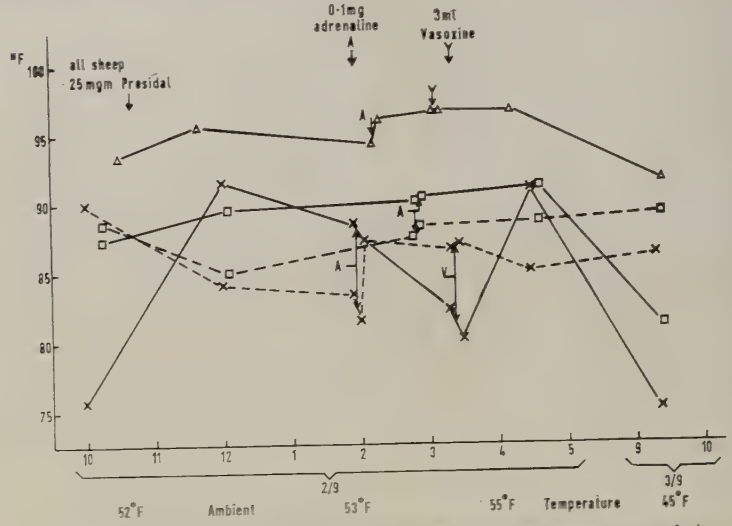

Fig. 8.—The effects of Presidal injection on the skin temperature of the side of the nose. (Sheep Nos. 7, 21, and 66.)

As indicated by the pupils, the Presidal was acting throughout the period of the trial. It appears to have increased the readings on the control side of the nose and decreased those on the sympathectomised side. This was very marked for sheep No. 7. The increase in skin temperature shown by sheep No. 66 could be the normal daily trend, but the subsequent drop in temperature suggests that the Presidal had caused a slight initial increase.

The results of adrenaline injection were inconsistent, while Vasoxine gave a response similar to that obtained without Presidal treatment.

After Shearing. Twenty-five mg of Presidal gave no response on the main body areas of sheep Nos. 7 and 66, but had effect on the

face skin temperatures of sheep No. 7 similar to that found before shearing. For this animal the control side rose from 72.2°F to 90.6°F and the sympathectomised side fell from 88.1°F to 83.6°F.

Reserpine

Before Shearing. Fig. 9 shows the effect of reserpine injection on the skin temperature of the nose (2.5 mg each injection).

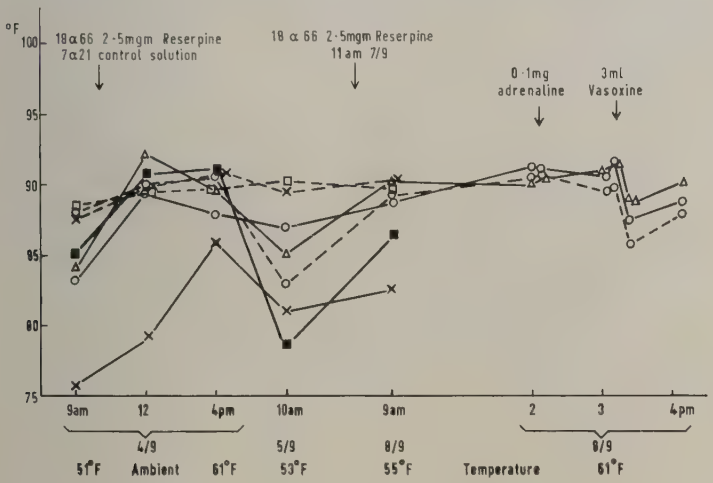

Fig. 9.—The effects of reserpine injection on the skin temperatures of the side of the nose. (Sheep Nos. 7, 18, 21, and 66.)

Although no tranquilising effect was seen until after the second injection, it appears that, by the day following the first injection, some effect on the blood supply was showing. This is indicated by a marked decrease in the temperature of the sympathectomised side of sheep No. 18 when compared with its normal values and those of the two control animals. Subsequently (8/9) temperature changes of the sympathectomised and control sides were very similar in their responses to adrenaline and Vasoxine. Sheep No. 66 did not depart from the normal daily temperature changes.

The depression in skin temperatures of the sympathectomised side is a result similar to that obtained with Presidal; and this, therefore, suggests that mechanisms other than the parasympathetic system may be concerned in this drop, as there is no evidence of effects of reserpine on cholinergic ganglia.

After Shearing. The final trial was made with reserpine, using the same dose rates as those used before shearing. Readings commenced after the second dose when the animals were tranquilised. Fig. 10 shows the results for the nose positions. Again, no skin temperature responses were found for the mid-side and shoulder positions. It is possible that an effect similar to that obtained before shearing

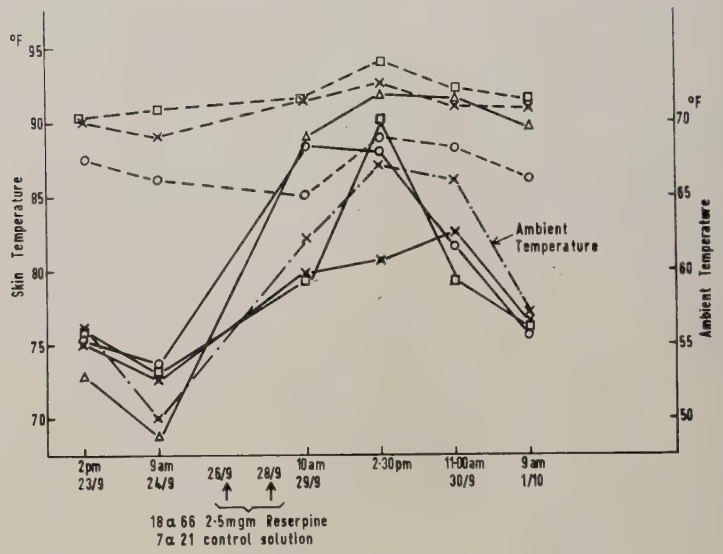

Fig. 10.—The effects of reserpine injection on the skin temperatures of the side of the nose after shearing. (Sheep Nos. 7, 18, 21, and 66).

(10 a.m., 29/9) occurred for the nose of sheep No. 18 in that the temperature of the control side was above that of the sympathectomised side.

The effect on sheep No. 66 is doubtful: it is confounded with the possible effect of rising ambient temperatures; also, its higher level of skin temperature was maintained.

Because of these confounding effects of rising ambient temperature and the lack of any means of controlling it in the available shed, no attempt was made to repeat these trials.

Discussion

Although a few tentative conclusions can be drawn from the results obtained with the various drugs, the first purpose of these trials was to obtain preliminary data on which to base the planning of future experiments. In addition, it was hoped to develop suitable techniques whereby blood supply might be altered over a period of time long enough to determine any associated effects on wool growth. A period of at least a month would probably be required in order to determine any effects on the slow-growing wool of the face region.

Given the knowledge obtained from these trials, it might then be possible to test the hypothesis that wool growth on the face is associated with the blood supply to the skin of that area. This hypothesis might be tested by two complementary methods: first, by skin temperature measurement of a reasonable number of sheep of differing

face cover; and, secondly, by a direct experimental approach which would involve changing the amount of the blood supply to the skin of a small number of animals.

For the experimental approach, allowances must be made for variations in individual behaviour which could be determined in an initial control period. Separate pens would be desirable so that readings could be taken in the pens with the minimum of disturbance. Some form of temperature control and freedom from draughts would be essential.

Sympathectomy appears to be the most efficient method of increasing the skin temperature of the face over a long period. This is in contrast with the results of a thoracic sympathectomy reported by Ferguson (1949), where little or no effect could be found a few months after the operation. This may have been the result of rising ambient temperature causing increased vasodilation on the control side or to regeneration of the sympathetic nerves. In addition, complete sympathectomy might be more difficult to achieve in the thoracic area. Sympathectomy also has the advantage of both a treated and a control side on one animal and the localising of the treatment to the required area. Unsympathectomised sheep should be included, as the results with ganglionic blocking agents suggest that the sympathectomy of one side may have some indirect effect on the control side. In addition to sympathectomy, treatments such as shearing could be of use in maximising the differences between the two sides and in determining the success of the operation.

The injection of drugs to test the effect on wool growth would be of doubtful use because of the generally short period of action compared with the long period required to test a wool growth response. Of the drugs tested, possibly Vasoxine in conjunction with sympathectomy could be of use if shedding of wool could be induced as a criterion of interrupted growth. Such a technique has been shown to be successful with mice in the anagen stage of hair growth (Cockrem 1959).

Ronicol could be of use in that the action was reasonably prolonged and probably confined to the "blush" area (Wertheimer *et al.* 1955). The remaining drugs, with sympathectomy, might be of use in determining the mechanisms involved if blood supply was shown to be relevant to the face cover problem. For such a purpose more extensive readings of blood pressure, body temperature, heart rate, and other variables would be necessary. However, results with ganglionic blocking agents can be variable and difficult to interpret (Hertzman 1959). This is to some extent true of the results obtained in these trials, but sufficient evidence of interest was obtained to merit a brief discussion of the possible factors involved in the control of the skin temperature of the side of the nose.

The most consistent observation was of a rapid rise in temperature of the control side, with a corresponding drop in temperature on the sympathectomised side, of the nose. This occurred with Vasoxine on sheep No. 7 and with the ganglionic blocking agents. The effect

of Vasoxine and of adrenaline might be explained by a greater sensitivity of the sympathectomised side and possibly by a difference in the dose leading to vasoconstrictor or vasodilator effects. However, the ganglionic blocking agents would be expected to lead to similar behaviour of the two sides, and, whereas this was true of the reserpine-treated sheep, it was the result of changes in the sympathectomised side rather than in the control side. Initially this could result from the release of adrenaline from the adrenal medulla (Eränko and Hopsu 1958), but such an effect should be followed by one associated with a lack of adrenaline in the circulation.

With Presidal, the parasympathetic system should also have been blocked; however, similar effects of a drop in the sympathectomised side and a rise in the control side were observed. These occurrences, together with the effects of acetylcholine in the Ronicol compositum, suggest that the parasympathetic, as well as the sympathetic, is involved in the control of blood supply to the face. Alternatively, generalised vasodilation could lead to a change in the balance of blood supply (De Bakey *et al.* 1947; Murphy, Haglin, and Felder 1957), and this is suggested by the fall in temperature of the sympathectomised side after reserpine treatment which was a similar effect to that of reserpine on the denervated rabbit's ear (McQueen, Doyle, and Smirk 1955). However, it is difficult to fit a rise in temperature on the control side into such an explanation. Further fundamental work on blood supply is required before a reasonable explanation can be given.

The dramatic drop in temperature after shearing on the control side of the nose may have resulted from the closing of arterio-venous anastomoses. Goodall (1955) has shown that the face and ears of the calf have numerous anastomoses in the first and second skin plexuses, while Beakley and Findlay (1955) have obtained sudden rises in the temperature of the ear with rising ambient temperatures. This sudden rise was attributed by them to the opening of anastomoses. In sheep Blaxter *et al.* (1959) found a similar dramatic rise in ear surface temperature at an ambient temperature of 60–70°F; these workers have suggested that this rise was also the result of the opening of anastomoses similar to those in the calf. If the drop in temperature on the control side of the face after shearing is the result of closing of such anastomoses, then the failure of the sympathectomised sides to show a similar drop would indicate sympathetic control of these arterio-venous shunts on the nose of the sheep. In addition, the relationship of the temperature of the control side with ambient temperature and the large drop after shearing suggests that the face region may be of importance to the control of heat loss in the sheep.

Ferguson (1949) obtained increased wool growth following sympathectomy when ambient temperatures were relatively low. Also Grant (1935) noted increased hair growth on the denervated rabbit's ear. While no detailed study of wool or hair growth was made during these trials, a patch was shaved on either side of the nose when the temperature readings were completed. Visual examination a fortnight later showed longer kemp growth on the sympathectomised side of two of the animals. This longer growth was particularly marked in the case of sheep No. 7, which had shown the greatest temperature difference between the two sides.

A further point of interest is that, after shearing, the lowest skin temperature in the main body area was in the lower mid-side position (Cockrem and Wickham 1960), where Henderson (1953) and Young and Chapman (1958) have found the rate of wool growth to be one of the lowest for the main fleece area. Although this might be partly explained in terms of heat generated by shivering in the underlying muscle, this does not explain some differences found for positions which should be similar in this respect. Henderson (1953) suggested that wool growth gradients might be associated with blood supply, but Ryder (1955) obtained no confirmation of this in the arrangement of the blood vessels found from anatomical investigation of sheep skins. Ryder's conclusion does not allow for the rate of blood flow depending on the size of the vessels, their distance from the heart, or possible differences arising from the physiological control of them. An investigation based on techniques of the type described in this paper could be a more accurate test of Henderson's hypothesis.

An association of skin temperature and wool growth, if it does exist, has three possible explanations:

(1) High skin temperatures are related to greater cutaneous blood flow. The supply of nutrients necessary for the physiological functions of the wool follicle originates from the blood stream. Hence an association between skin temperature and wool growth could be a result of a better supply of nutrients reaching the follicle.

(2) The rate of diffusion of nutrients across the follicle membranes, from the capillaries to the position where these nutrients play their part in fibre formation, could be a factor limiting wool growth. Diffusion rates, in general, tend to increase at higher temperatures.

(3) The speed of chemical reaction is influenced by temperature. It is possible that the rates of the biochemical processes involved in the conversion of substrate into wool fibre are more important in limiting output than the supply of substrate. A higher skin temperature could permit a faster rate of reaction.

Thus it can be seen that a relationship between skin temperature (or cutaneous blood flow) and wool growth need not necessarily indicate that the supply of nutrients to the follicle via the blood stream is the factor limiting wool fibre output, although the temperature factors would themselves be partially dependent on the blood supply.

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A COMPARISON OF BARLEY MEAL, MEAT MEAL, AND DRIED BUTTERMILK, FED ALONE AND IN VARIOUS COMBINATIONS, AS SUPPLEMENTS TO WHEY FOR FATTENING PIGS

By A. C. Dunkin, Massey Agricultural College, Palmerston North

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Summary

Two trials are reported in which pigs were fed at liveweights starting at 60 lb and 50 lb respectively, and finishing at 110 lb, with whey supplemented with meal mixtures containing a variety of proportions of barley meal, meat meal, and dried buttermilk.

In the first trial, a daily meal supplement consisting of 1 lb barley meal gave growth as rapid as a supplement consisting of ½ lb meat meal and ½ lb barley meal. Complete replacement of the barley meal with meat meal resulted in a 15% reduction in growth rate and food efficiency; the difference in efficiency was significant ($P<.05$), while the difference in growth rate just failed to reach significance. A supplement consisting of ⅜ lb dried buttermilk and ⅝ lb barley meal resulted in significantly faster and more efficient growth than the meat meal supplement ($P<.01$).

In the second trial, a 15% higher level of feeding was employed. Initially the daily meal supplement was 1½ lb; after the attainment of 80 lb liveweight the supplement was reduced to 1 lb. Seven supplemental meal mixtures were compared, consisting of barley meal alone, and ¼ lb, ½ lb, and 1 lb of either meat meal or dried buttermilk, together with appropriate amounts of barley meal. Relative to the all-barley supplement, none of the supplements containing meat meal produced any significant difference in performance. However, growth rate and food efficiency improved progressively with increasing proportions of dried buttermilk in the supplemental mixture.

Introduction

In New Zealand, the use of concentrates in pig feeding is restricted mainly to the supplementation of diets based on liquid dairy by-products. The range of concentrates available for this purpose is comparatively small, the commonest being barley meal, meat meal, and dried buttermilk. Although these differ widely in nutritive content and balance, there is little uniformity of practice in their use in supplementing dairy rations for fattening pigs.

In so far as whey supplementation is concerned, whereas some farmers use barley meal, others use only dried buttermilk. Various combinations of barley meal with dried buttermilk or meat meal are also fed. Some farmers still adhere to the use of meat meal which, 25 years ago, was very commonly employed as the sole supplement

because dried buttermilk was not then available and barley meal was much more expensive than meat meal.

In view of the relatively low protein content of whey, it is usually considered necessary to include a protein-rich concentrate in the ration in order to achieve satisfactory growth. In trials with fattening pigs in England, in which unrestricted quantities of whey were supplemented with a daily meal allowance of 3 lb, decreasing to 2 lb when the pigs were 13 weeks old, it was suggested that the inclusion of 10% fishmeal in the supplement represented about the minimum level of protein concentrate likely to permit satisfactory growth (Braude *et al.* 1958).* In New Zealand, however, for economic reasons, daily meal allowances are limited in commercial practice to no more than 1–1½ lb per pig. For these conditions, too, Peirson (1949) has recommended that a protein concentrate should be included. However, as far as can be ascertained, no recent work has been reported concerning the composition of meal mixtures suitable for supplementing whey when the daily meal allowance is restricted to 1–1½ lb. It may be that, when concentrates are used as sparingly as this, growth rate is limited, not by insufficent protein, but by the level of energy intake that can be achieved in this excessively bulky diet.

The object of the trials reported here was to obtain information on the relative suitability of various combinations of the three concentrates most commonly used for this purpose in New Zealand, when limited in amount to 1–1½ lb per pig daily.

Method

A preliminary trial, carried out in the autumn of 1958, was followed by a larger trial during the period September 1958 to February 1959.

Experimental Design and Animals Used

Both trials were of randomised block design; the first consisted of four treatments each with six pigs; the second consisted of seven treatments, each with four pigs, in each of two successive sub-trials. In both trials first-cross (Large White x Berkshire) pigs, which had been weaned at three weeks of age, were used. Experimental blocks consisted of litter-mates, matched for weight and previous growth rate. Allocation of litter-mates to treatments was at random save for the restriction that, as far as possible, the treatment groups were balanced for sex. This was achieved except in the case of Treatment 2 of Trial 1 (two sows and four barrows) and Treatment 2 of Trial 2 (three sows and five barrows).

Experimental Treatments

Trial 1. In the first trial a comparison was made between the following meal supplements; in each case the supplement was fed at the rate of 1 lb per pig daily.

* This is supported by the results of a further trial that has since been reported by these authors. (Braude, R.; Mitchell, K. G.; Cray, A. S.; Franke, A.; Sedgwick, P. H. 1959: *J. Dairy Res. 26:* 238).

Treatment 1—Barley meal alone and minerals (1 oz per pig daily of a mineral mixture consisting of 4 parts boneflour: 1 part common salt, to compensate for the low calcium and salt content of barley meal).

Treatment 2—Equal parts meat meal and barley meal.

Treatment 3—Meat meal alone.

Treatment 4—Two parts dried buttermilk and one part barley meal.

Whey was rationed according to the scale given in Table 1. This scale was derived from one reported by Smith (1956) as having given maximum efficiency of food conversion from 40–80 lb liveweight of pigs on a separated milk diet, by substituting 15 lb whey or 1 lb meal for each gallon of separated milk.

TABLE 1. Scale of Whey Feeding Used in Trial

Live Weight (lb)	Daily Whey Ration (lb)
46–50	15
66–70	24
86–90	32
106–110	39

(Intermediate values were obtained by interpolation.)

Trial 2. In the second trial, the number of combinations of the three concentrates was extended. Furthermore, the level of feeding —in terms of daily dry matter (d.m.) intake—was raised by approximately 15% compared with that of Trial 1. The object of raising the level was to discover whether the rations containing the lower amounts of protein would prove less satisfactory than the remainder when the overall plane of feeding was raised. In order to achieve this higher level of food intake, in the earlier stages of growth the daily meal allowance was set initially at 1½ lb. After 65 lb liveweight this was

TABLE 2. Scale of Meal and Whey Feeding Used in Trial 2

Liveweight (lb)	Meal (lb per day)	Whey (lb per day)
40	1.5	12
50	1.5	16
60	1.5	19
70	1.4	25
80	1.0	36
90	1.0	39
100	1.0	43
110	1.0	47

reduced gradually to a minimum of 1 lb at 80 lb liveweight. The daily meal and whey allowances fed are shown in Table 2.

The composition and daily allowances of the various meal mixtures used in the seven experimental treatments are listed below, the requisite reduction in quantity from 65 lb onwards being effected by gradually eliminating ½ lb of barley meal from each of the mixtures.

Treatment 1—Barley meal alone (1½ → 1 lb)*.
Treatment 2—¼ lb meat meal + barley meal (1¼ → ¾ lb)**.
Treatment 3—½ lb meat meal + barley meal (1 → ½ lb).
Treatment 4—1 lb meat meal + barley meal (½ lb → nil).
Treatment 5—¼ lb dried buttermilk + barley meal (1¼ → ¾ lb)**.
Treatment 6—½ lb dried buttermilk + barley meal (1 → ½ lb)**
Treatment 7—1 lb dried buttermilk + barley meal (½ lb → nil).

* 1 oz per pig daily of mineral mix (4 parts boneflour: 1 part common salt).
** ½ oz per pig daily of mineral mix.

Composition of the Foods

Different consignments of the concentrate meals were used for the two trials. The percentage compositions of samples of the foods are given in Table 3. Only d.m. and crude protein figures are available for food employed in the first trial.

The whey used was derived from Cheddar cheese manufacture. It was sampled daily, d.m. determinations being carried out on weekly composite samples.

TABLE 3. Percentage Composition of the Foods

Food	Trial	Dry Matter		Crude Protein (Nx6.25)	Ether Extract	N.-Free Extract	Crude Fibre	Ash
Meat Meal	1	91.6		58.6				
	2	90.7		56.0	9.8	1.4	—	23.5
Dried buttermilk (roller dried)	1	96.1		36.1				
	2	93.2		31.6	6.1	48.1	—	7.4
Barley Meal	1	84.0		11.9				
	2	83.5		11.7	1.3	63.7	4.1	2.7
			s.d.					
Whey	1	6.1	0.46					
	2a	6.4	0.1					
	2b	6.3	0.21					

s.d. = standard deviation of individual observations.

Pre-Experimental Feeding and Management of Pigs

In Trial 1, the 24 experimental animals were selected at approximately 12 weeks of age, when their average weight was 55 lb

(range 46–61 lb). Six pigs were housed in each of four pens in a double-sided, uninsulated fattening house with external dung passages. Each pen contained 1½ blocks of pigs. The pigs were fed individually. At this stage, all pigs were receiving 1½ lb meal daily (equal parts barley and meat meal) plus whey. The pigs were all brought on to trial simultaneously after nine days. During the latter half of this period, the experimental meal supplements were gradually introduced.

For each of the two sub-trials comprising Trial 2, 28 pigs were selected at 8 weeks of age and moved to a well-insulated totally enclosed piggery of the Danish type, equipped with an air-conditioning unit. The mean air temperature, as recorded by thermograph, located at a height of 5 ft above the pen floor, was 69°F. The range 64°–72°F was exceeded for only 6% of the time in sub-trial A and for 10% of the time in sub-trial B (maximum range 62°–80°F). Feeding consisted of 1 lb barley meal, ¼ lb meat meal, and ¼ lb dried buttermilk per pig daily, plus whey fed according to scale. At 43–45 lb, the pigs were placed in separate pens and the meal allowance was changed gradually to the appropriate experimental mixture. Pigs were placed on trial individually as they reached 48–52 lb liveweight.

General Management

In Trial 1, whey was at first fed three times daily, the concentrates being given prior to the morning and evening feeds. However, when it was found that a considerable number of pigs were not clearing up their rations, the midday feed was replaced by two feeds, one at 10 a.m. and one at 2 p.m. In Trial 2, where the pigs had constant access to their troughs, thrice-daily feeding was satisfactory.

Once weekly all pigs received 5g of an emulsion containing 5,000 i.u. Vitamin A and 500 i.u. Vitamin D_3 per g. No bedding was provided, but in the case of Trial 1 sleeping areas were fitted with raised wooden floors and with hardboard hovers lined on the underside with aluminium foil.

The pigs were weighed twice weekly before the morning feed throughout both trials and the individual rations altered in accordance with the feeding scales. All whey refusals were weighed but meal refusals were estimated.

Observations on the consistency of the dung of individual pigs were made once daily, using the same subjective classification as that employed in an earlier trial (Dunkin 1959).

In both trials, the experimental treatments were continued until all pigs weighed at least 110 lb liveweight.

Biometrical Treatment of Data

In the case of casualty pigs whose results were discarded, appropriate missing plot values were calculated from the remaining data.

For the purposes of analysis, rates of gain and food economy were assessed respectively in terms of the number of days from the start of the trial until 110 lb liveweight was reached, and the total amount of meal, whey, and d.m. consumed during this period. In Trial 1, because the experimental treatments were started simultaneously for all pigs, analyses of co-variance were undertaken with initial liveweight as the independent variable. The results of the two successive sub-trials comprising Trial 2 were pooled for analysis, the variance between blocks including the variance between sub-trials, since a preliminary analysis showed the latter variance to be very small.

For testing the significance of differences between the individual treatments the multiple range test of Duncan (1955) was used.

Results

Trial 1

A summary of the mean results for growth rate, food consumption, and economy of gains for the four treatment groups is presented in Table 4.

TABLE 4. Trial 1—Treatment Means for Growth, Food Consumption, and Efficiency of Food Conversion from Approximately 60 lb to 110 lb Liveweight

Treatment	1	2	3	4	Sign. of Diff.	Error Coeff. of Variation
Daily meal suppl.	1 lb barley m.	½ lb meat meal ½ lb barley meal	1 lb meat meal	⅔ lb b/ milk ⅓ lb barley meal		
Initial livewt. (lb)	60.5	60.8	61.5 †	61.0		
Days to 110 lb	58.0	58.5	66.2	52.3	T.4<T.3**	11.5%
Daily gain (lb)	0.85	0.84	0.73	0.94		
Meal (lb)	58.0	58.5	62.2	52.3	N.S.	11.4%
Whey (lb)	1,701	1,718	1,922	1,517	T.4<T.3**	11.8%
Total d.m. (lb)	152.6	156.1	174.9	140.5	(T.4<T.3** (T.1<T.3*	11.5%
Meal/1 lb gain (lb)	1.17	1.18	1.28	1.10		
Whey/1 lb gain (lb)	34.3	34.7	39.5	31.0		

† Missing plot values calculated for one pig.
* P<.05.
** P<.01.

Although a relatively restricted scale of feeding was employed, several pigs consistently refused some of their whey. Some treatment differences in this respect were noted, the average whey refusals for Treatments 1–4 amounting to 2.7%, 0.6%, 0.7%, and 3.5% respectively of the total whey offered. Particularly at the

start of the trial several of the Treatment 3 pigs found their all-meat meal supplement unpalatable, and three of the six pigs in this group each refused an estimated total of 6–8 lb (10–12%) meat meal over the course of the trial.

Growth rate was erratic in the case of several pigs, the rates of gain of no less than six animals being appreciably less than those of their treatment mates. The results were discarded for one pig on Treatment 3, which stopped growing at about 100 lb liveweight. In spite of this variability the relative performance of pigs on the various treatments was very consistent. Thus, in five blocks out of the six, the pig fed buttermilk and the pig fed 1 lb meat meal had the fastest and the slowest growths respectively.

In all co-variance analyses the regressions of time to reach 110 lb liveweight, and total meal, whey, and d.m. consumption on initial liveweight, were non-significant. Those pigs receiving buttermilk in their meal supplement grew significantly faster and more economically than those on the all-meat meal supplement. The all-barley meal supplement group also required significantly less food than those pigs on the all-meat meal supplement, while the difference in fattening time just failed to reach the 5% level of significance.

In general, the incidence of scouring was unduly high in view of the restricted level of feeding, becoming increasingly widespread from 75–90 lb liveweight onwards. There was some indication that scouring occurred most frequently on the buttermilk supplement, but a satisfactory comparison between treatments was not possible because of the difficulty of obtaining an adequate number of observations on individual pigs where members of different treatment groups were penned together.

Trial 2

In sub-trial A, one pig on Treatment 4 (1 lb meat meal) which had grown normally until 95 lb liveweight, subsequently made only slight and erratic gains for several weeks. A course of sulphamezathine injections had no apparent effect, and, as this pig had not reached 110 lb liveweight by the time the trial ceased, its results were discarded. Two other pigs, which were off their food for a few days, were treated with sulphamezathine and recovered rapidly. In sub-trial B, one pig on Treatment 3 (½ lb meat meal) suddenly became severely paralysed in the hind legs and had to be removed from the trial. All other pigs remained in good health.

The mean growth curves for the seven treatment groups are presented in Fig. 1 and a summary of the results for growth, food consumption, and efficiency of food utilisation in Table 5.

Those pigs fed the supplement containing 1 lb buttermilk grew significantly faster and more efficiently than those on all other treatments, while those receiving lesser amounts of buttermilk had significantly better food utilisation than the pigs given the 1 lb meat meal supplement. The results for the remaining treatments were very similar to each other.

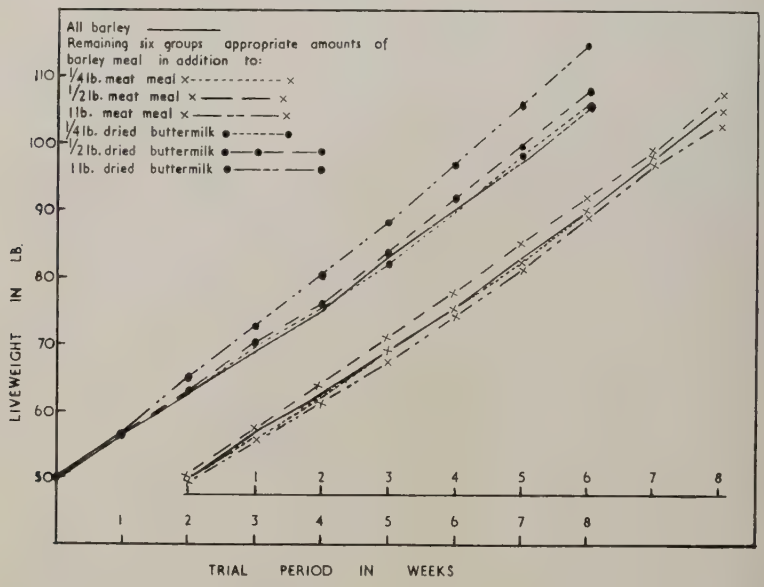

Fig. 1.—Average growth curves for each of the seven experimental groups of Trial 2. All groups received a daily meal supplement of 1½ lb per pig until 80 lb liveweight, and 1 lb thereafter.

In order to test the existence of any linear relationship between the level of protein-rich foods in the supplement and fattening time, regressions were calculated on the three buttermilk treatments and on the three meat meal treatments, the independent variable being the daily allowance of these foods. In the case of the buttermilk treatments, a highly significant regression was obtained ($F = 49.9$, d.f. 1, 15), leading to the equation $Y = -9.12X + 60.8$ where $Y =$ the number of days to grow from 50–110 lb liveweight and $X =$ the daily allowance of buttermilk in lb. A significant negative regression of total consumption of d.m. on level of buttermilk fed was also found.

In contrast with these results, there was no significant trend ($F = 0.66$, d.f. 1, 13) among the meat meal treatments in respect of fattening time but total d.m. consumption increased significantly with increasing meat meal in the ration ($P<.01$).

The incidence of scouring was generally low, with no obvious treatment differences. Thus the average percentage of "scour" observations on any treatment ranged only from 2.6% to 12%.

DISCUSSION

The food refusals and the erratic growth of some pigs were suggestive of a virus pneumonia infection. As would be expected, the higher plane of feeding and the better environmental conditions

TABLE 5. Trial 2—Treatment Means for Growth, Food Consumption, and Efficiency of Food Conversion from 50 lb to 110 lb Liveweight

Included in Daily Meal Supplement	Treatments							Significance of Difference	Error Coeff. of Variation
	1 All Barley M.	2 ¼ lb Meat M.	3† ½ lb Meat M.	4† 1 lb Meat M.	5 ½ lb B/milk	6 ½ lb B/milk	7 1 lb B/milk		
Days to 110 lb	59.6	60.0	58.8	61.0	58.1	56.9	51.5	T.7<T1, 2, 3, 4, 5, 6** T.6<T4*	4.9%
Daily gain (lb)	1.01	1.00	1.02	0.98	1.03	1.05	1.17		
Food consumed:— Meal (lb)	73.9	74.6	72.6	75.1	71.8	70.3	63.8	T7<T1, 2, 3, 4, 5, 6** T6<T2, 4*	5.1%
Whey (lb)	1,754	1,755	1,766	1,804	1,713	1,663	1,500	T7<T1, 2, 3, 4, 5, 6** T6<T2, 4*	5.7%
Total d.m. (lb)	174.0	175.7	175.4	182.5	170.8	167.7	154.1	T7<T1, 2, 3, 4, 5, 6** T6<T4** : T5<T4*	5.3%
Efficiency of food conversion:									
lb meal/1 lb l.wt. gain	1.23	1.24	1.21	1.25	1.20	1.17	1.06		
lb whey/1 lb l.wt. gain	29.2	29.3	29.4	30.1	28.6	27.7	25.0		
lb d.m./1 lb l.wt. gain	2.90	2.93	2.92	3.04	2.85	2.80	2.57		
Estimated lb T.D.N./ 1 lb l.wt. gain	2.50	2.53	2.52	2.60	2.51	2.49	2.35		

† Missing plot values calculated for one pig.
* P<.05.
** P.<.01.

in Trial 2 resulted in a general improvement in growth rate and a reduction in within-treatment variability compared with Trial 1. However, the picture that emerges from the results of both trials is similar. Bearing in mind the differences between the trials with regard to housing and the planes and systems of feeding adopted, this consistency gives added weight to the treatment trends that were obtained.

The most notable feature was that in neither trial did the substitution of more meat meal or less meat meal in the barley meal supplement produce any improvement in performance. On the contrary, there was a tendency for the highest level of meat meal to depress growth rate and food economy. This effect was especially marked in the first trial, where the pigs fed the meat meal supplement grew only 85% as fast as those given the barley meal supplement. Presumably this was partly due to the fact that some pigs did not relish the meat meal and did not eat their full ration. However, the amount involved was small, and, since whey consumption was not affected, this factor alone would not account for the significant depression of performance that occurred. It seems more likely that the digestible energy content of this particular consignment of meat meal was less than that of the barley meal.

The relatively better growth of the pigs fed 1 lb meat meal in Trial 2 could be explained by differences in relative nutritive value between the different consignments of meat and barley meal used respectively in the two trials. The absence of any reluctance to eat the 1 lb meat meal supplement in the second trial could have been due either to the fact that in the early stages it was mixed with ½ lb barley meal—which helped to disguise its unpalatability—or that this consignment was more palatable.

It may not be entirely without significance that most of the ill-health and subnormal growth occurred on the treatments in which a high proportion of meat meal was fed. All three casualty pigs whose results were discarded had been fed supplements containing ½ or 1 lb meat meal; while, in Trial 1, of the six pigs that grew appreciably slower than the general average, three were from the group fed 1 lb meat meal.

In view of the failure of the addition of meat meal to improve on a cereal supplement for whey feeding, it is of interest to compare the estimated average daily intakes of crude protein by pigs on the several treatments of Trial 2 with those reported by Woodman and Evans (1945) for an all-meal ration which was thought to contain about the minimum level of protein sufficient to permit optimum growth. In Fig. 2 are presented graphically the data from Trial 2, together with those calculated from figures given by these authors for a ration containing 7% fishmeal until 90 lb liveweight and no protein concentrate thereafter. For the Trial 2 data the crude protein content of whey was taken as 0.9%.

It will be noted that, up to the 90 lb stage, the average daily protein intakes on the supplements containing barley meal, ¾ lb and ½ lb dried buttermilk, and ¼ lb meat meal, are all lower than the

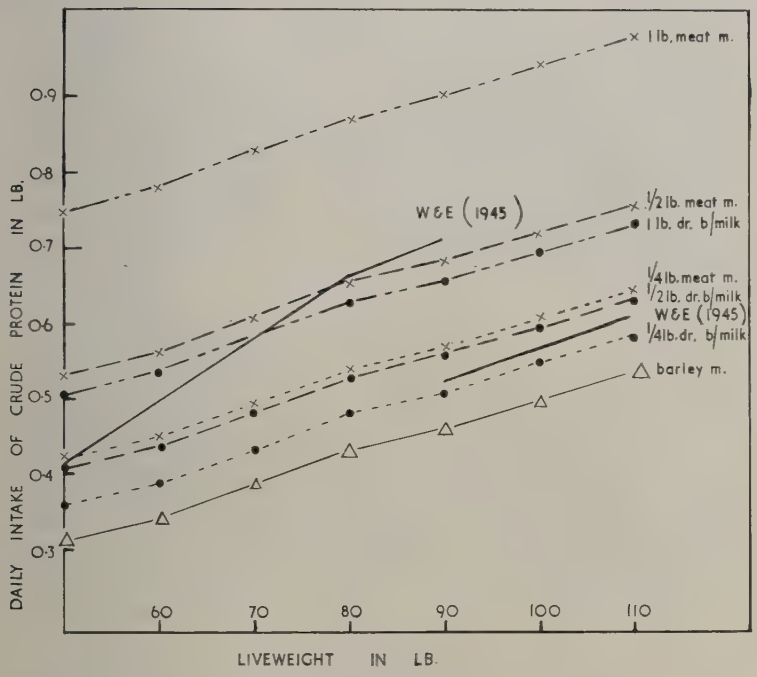

Fig. 2.—Daily intake of crude protein in relation to liveweight for each of the seven rations used in Trial 2 and for an all-meal ration used by Woodman and Evans (1945).

intake of the all-meal ration containing 7% fishmeal; and that, subsequently, the intake of the barley meal supplement continues well below that of the all-meal ration. However, the all-meal ration represented a level of feeding approximately 17% higher than that employed in Trial 2, where the excessive bulkiness of the whey necessitated the adoption of a more restricted scale of feeding. It seems therefore that, as the level of feeding is reduced, a lower intake of protein and a wider nutritive ratio may be adequate. The fact that whey protein is of high biological value (Wegelin 1952) may also be a mitigating factor.

The positive response to the substitution of dried butter milk for barley meal in the meal supplement contrasts with the negative results obtained when the barley meal was replaced by meat meal. It seems probable that much of the superiority of the buttermilk was due to its higher energy content compared with that of the other two foods. Thus, over the liveweight range 50–110 lb, it is estimated that the supplement containing 1 lb dried buttermilk supplied an average of 9% more total digestible nutrients (t.d.n.) daily than the barley meal supplement. It will be noted from Table 5 that, when total food intake is expressed in terms of estimated

t.d.n., rather than as d.m., differences between treatments are reduced considerably. In order to determine the extent to which the observed superiority of dried buttermilk is due exclusively to its high energy content, it would be necessary to carry out further trials in which the various supplements containing buttermilk are compared on an equal-energy basis. There is the further possibility that at least some of the observed differences between the supplements containing buttermilk and those containing meat meal were due to the protein of the meat meal being of relatively low nutritive value. Work reviewed by Duckworth (1955) has revealed considerable variation between different samples of meat meals in this respect.

The results obtained suggest that, for supplementing whey, where the daily meal allowance is limited to only 1–1½ lb of barley meal, a shortage of protein is unlikely to limit growth. At this comparatively low level of meal feeding, it seems that the choice of the meal ingredients should be determined primarily on the basis of relative cost per unit of energy content. Nevertheless, because minimum protein requirements are likely to vary with different conditions, and because the present trials did not start until 50 lb liveweight, it would be a wise precaution to include a little protein-rich meal in the ration in the early stages of growth, say, up to 70 lb liveweight. The results reported here suggest that for this purpose dried buttermilk is better than meat meal.

Acknowledgments

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STAPLE STRENGTH

II. SOME FACTORS AFFECTING THE MEASUREMENT OF STAPLE STRENGTH

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Summary

The effect of varying test length on staple strength was investigated.

The relation of fibre length to test length was studied; the interpretation of staple breaking graphs in terms of fibre length distribution and of fibres of primary and secondary follicle origin is discussed.

Considerable variation in staple strength over the body of Romney ewes was found. Samples from the neck were most sound, being very much stronger than samples from other body regions in one group of ewes which had a very severe "lambing-break". The effects of weathering on staple strength measurements are discussed.

A coefficient of variation of approximately 15% was found for the variation in maximum load or work between staples within samples. The variation was lower with sound wools than with tender wools.

Introduction

The general method developed for measuring staple strength, together with the results of tests on Romney and Corriedale wool which illustrate the type of results obtained, was given in Part I of this series (Ross 1960a)*. Data were also presented on some factors which affect this measurement, and also on its relation to a subjective hand assessment of the severity of the break.

This technique of measuring staple strength was applied to measurement of the "lambing-break" that is found in post-lamb shorn fleeces about a third of the staple length up from the butt. As a result of the normal seasonal variations in fibre diameter (Story and Ross 1960), together with the additional stresses of pregnancy and lactation, the measurement of staple strength at this level will give the minimum strength for most staples. Breaks, however, sometimes occur at other levels in the staple, or the fibres may be weakened due to weathering.

Because of the very nature of a wool staple and of the fleece, there are a large number of factors which may affect any estimate

* The method and results were first presented at the 32nd A.N.Z.A.A.S. meeting at Dunedin, January 1957.

of the composite strength of the individual fibres. This paper deals with some of the major factors which will affect the measurement and interpretation of staple strength results.

The Effect of Test Length on Staple Strength

General

The value found for the strength of a staple in lb/g will depend primarily upon the distance apart of the clamps, the position on the staple at which the clamps are placed, and the strength of the fibres between the clamps. Even with a perfectly sound staple in which the fibres have a constant breaking stress along their length, the maximum load in lb/g at different positions on the staple for a given test length will not be constant due to variation in cross-sectional area along the fibre, which will alter the relation between the total of the minimum cross-sectional areas of the fibres and the total fibre weight at the given test length. This affects the results of staple strength tests on pre-lamb and post-lamb shorn staples, where the mean minimum diameter of the fibres between the clamps was much lower for post-lamb shorn than for the pre-lamb shorn staples (Ross 1960a).

The upper limit of the test length will be determined by the crimped length of the shortest fibre of full years' growth in the staple. For comparative tests on staples from different sheep, or different positions on individual sheep, the maximum test length will also depend upon the length of the shortest staple; weathered tips should be avoided. In most staples the region of lowest tensile strength is just above the butt in the "winter-fine lambing-break" region. If the butt 0.75 in. of a Romney staple is held in the lower clamp, the break region in post-lamb-shorn wool will usually be within the test length. With shorter Halfbred or Merino staples, however, the break is often so close to the butt that it will be covered by the butt clamp, thus resulting in a misleading value (which is high) for staple strength.

From tests with equal bundles of viscose rayon fibres which were relatively uniform in fibre dimensions, it was found (Ross 1960b) that, as the test length increased, the actual bundle breaking load decreased linearly. While a part of this decrease in strength was due to the increase in breaking time as the test length increased, it was principally due to the increasing chance of including a weak region between the clamps as the test length was increased.

Experimental Procedure

Ten Romney wool samples were selected which were known, from earlier tests at a test length of 2.5 in., to cover the normal range of staple strengths. Two staples were broken from each sample at each of seven test lengths varying in 0.5 in. steps from 1 in. to 4 in.; in all cases the butt 0.75 in. of the staple was clamped in the lower jaw.

Results

Maximum Load. As the variation in strength between staples within each sample was quite high, some irregularity was shown by the results. The mean values for maximum load (lb/g) are given in Table 1. The samples are arranged from 1 to 10 in

TABLE 1. THE RELATION BETWEEN MAXIMUM LOAD AND TEST LENGTH

Test Length (in.)	Sample No. 1	2	3	4	5	6	7	8	9	10
	Maximum Load lb/g									
1.0	540	496	459	262	332	283	338	251	139	226
1.5	366	332	321	173	166	190	99	109	41	52
2.0	298	183	209	172	142	150	78	72	32	42
2.5	220	201	181	125	107	100	83	46	25	24
3.0	179	153	100	113	103	72	53	36	9	14
3.5	134	100	71	69	89	67	53	32	12	11
4.0	121	98	42	26	84	58	30	37	6	11
	Maximum Load lb/g × Test Length									
1.0	540	496	459	262	332	283	338	251	139	226
1.5	549	498	482	260	249	285	149	164	62	78
2.0	596	366	418	344	284	300	156	144	64	84
2.5	550	503	453	313	268	250	208	115	63	60
3.0	537	459	300	339	309	216	159	108	27	42
3.5	469	350	249	242	312	235	186	112	42	39
4.0	484	392	168	104	336	232	120	148	24	44

decreasing order of their maximum load at 2.5 in. In general, the order of maximum load values was similar at other test lengths to that encountered in the sequence measured at 2.5 in. Divergence from this sequence was most marked at test lengths of 1.0 in. and 4.0 in. The decrease in strength at 1.0 in., while greater for the very weak samples 9 and 10 than for the sound staples 1 and 2, was also higher for samples 3 and 4—which had a definite tender region above 2.5 in.—compared with samples 5 and 6, which, although of only medium strength, were not tender at a particular level in the staple. Also given in Table 1 are the values for minimum load × test length. These results give a clearer picture of the effect of variation in test length on staple strength than the maximum load figures alone.

A decrease in strength would be expected with increasing test length even in very sound staples such as samples 1 and 2, in which maximum load × test length was approximately constant from 1 in. to 3.0 in., decreasing thereafter. Samples 5 and 6 were also sound throughout, with no obvious break, but they were of considerably lower strength than samples 1 and 2. Samples 3 and 4 were sound until test lengths of 3.0 in. and 3.5 in. respectively were

reached, after which weak regions in the staples were included in the test length, resulting in a large decrease in staple strength. In samples 7–10 there was a break between 1.0 in. and 1.5 in. This resulted in a marked fall in strength, which decreased thereafter only slowly with increasing length. No doubt there had also been some reduction in the strength of the fibres clamped at 1.0 in., as in sample 9. No data, however, are yet available on the effect of a stress causing either a break or a serious weakening of strength in wools which were initially of known degrees of soundness. In the regions above the break, samples 7–10 may have been equally as strong as samples 1 and 2.

Work. With increasing test length, the actual amount of work in cm lb required completely to break the staple increased; however, in cm lb/g, it decreased, but at a slower rate than the decrease in maximum load. The work results in general followed the maximum load results, although for a similar cross-sectional area of fibre the results (cm lb/g × test length) were somewhat different. Fig. 1 shows these data for seven of the samples, the other three being omitted to avoid confusion. For samples 1 and 5,

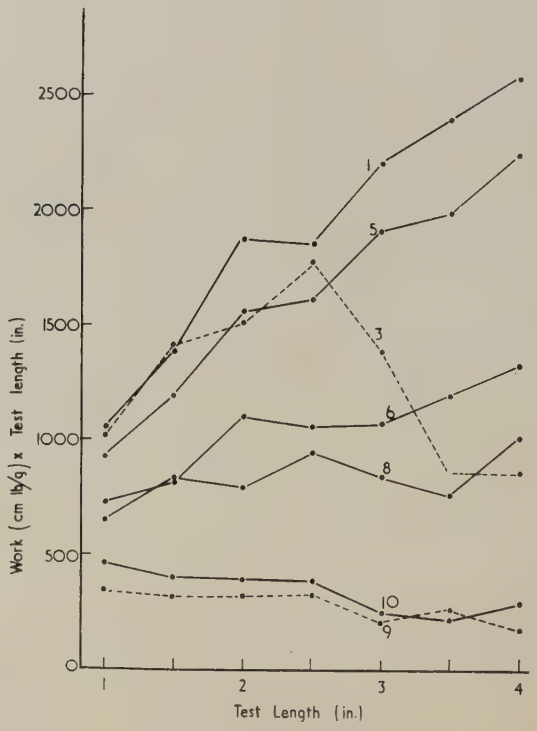

Fig. 1.—Relation between test length and work (cm lb/g) × test length, for wools of varying degrees of soundness.

work × test length increased at an approximately constant rate as test length increased. The extension of sample 5 was much higher than that of sample 1, resulting in a much smaller difference in the work required completely to break these samples than would be indicated by the difference in maximum load. Sample 3 showed a similar increase up to the weaker region above 2.5 in.; thereafter work decreased rapidly to a test length of 3.5 in. Samples 6 and 8 showed a small rise with increasing test length, while the very weak samples 9 and 10 showed a decrease as the test length was increased.

Extension. As the test length increased, so the extension at maximum load and at staple break increased. The average extension at maximum load increased from 0.65 in. at a test length of 1.0 in. to 1.94 in. at 4.0 in., which was equivalent to a decrease of from 65% to 49% of the test length. Similarly the mean extension at break increased from 1.61 in. at 1.0 in. to 3.66 in. at 4.0 in., equivalent to a decrease of from 161% to 91%. While the traverse results were reasonably consistent for all samples the extensions at maximum load showed more variation, particularly in the weaker samples. The breaking angle also decreased as the test length increased.

Discussion

These results illustrate that, to interpret staple-strength results, the position of the clamps on the staple relative to the butt, and the region of lowest tensile strength, must be known. The calculation of strength tests to a similar cross-sectional area of fibre, maximum load or work/g × test length, while assisting in the interpretation of strength tests at different lengths on individual samples, would not be a satisfactory basis for the comparison of tests on various samples at different test lengths, as is evident from Table 1.

Even in sound samples, maximum load lb/g decreased with increasing test length at a greater rate than could be accounted for by changes in fibre weight alone. On the other hand, the work required completely to break a similar cross-sectional area of sound staples increased with increasing test length due to the increase in absolute extension at the longer test length. Due to differences in extensibility of wools, similar mean maximum load values may be associated with varying amounts of work.

The Relation between Fibre Length and Test Length

General

With test length as a constant, the mean fibre length between the clamps will depend on the number and depth of the crimp waves of the individual fibres. A staple in which all the fibres were uniform in length, diameter, strength, and extensibility would extend and break as a unit, resulting in a higher maximum load than that of a staple in which the fibres were of similar diameter, strength, extensibility, and mean fibre length but in which there was a wide

distribution of fibre lengths. The total work required to break these two staples would however be similar.

Lang and Chaudhri (1948) found that the mean fibre length of 20 Merino staples was 1.37 × the staple length, while the coefficients of variation of fibre length ranged from 8.6% to 17.6%, with a mean of 11.7%. Goldsworthy and Lang (1954) found that the mean fibre length of 1-cm sections of staples varied both along individual staples and between staples, and ranged from 1.04 cm to 1.41 cm, with a tendency for the mean fibre length to be longer with finer wools. Doney (1959) found mean values for the ratio of fibre length to staple length, of five Merino ewes and five Border Leicester × Merino ewes, of 1.46 and 1.45 respectively.

As the test length used for staple strength measurements does not include the tip region, lower values might be expected for the fibre-length/test-length ratio than for the fibre-length/staple-length ratio.

Within a Romney Crossbred wool staple there is a high positive correlation between fibre diameter and fibre length. If the coarsest fibres at a given test length have the longest fibre length, these fibres will be the last to extend and rupture during staple strength measurement. Therefore, some knowledge of the relation between fibre length and both mean and minimum fibre diameters is basic to the interpretation of staple strength measurements.

Experimental Procedure

Thirteen sound staples were selected which had well-defined crimps ranging from 1.7 to 14.4 crimps/in. The staples were clamped as for breaking at 2.5 in. (6.35 cm), and the fibres cut from between the clamps. An average of 249 fibres was measured for fibre length from each staple on a W.I.R.A. fibre length machine. After being measured, the fibres were combined into groups differing in mean length by 1 cm, cleaned by extraction with petroleum ether followed by a warm water wash, dried to a low regain, then conditioned at 68°F and 65% R.H. and weighed. The mean fibre diameter for each length group and for the whole sample was then calculated from these results. On the average there were about 2% of short fibres in the samples less than 6.0 cm long, due either to fibre breakage or shedding. These fibres were not included in the results.

Results and Discussion

As shown in Table 2, there was no relation between mean fibre length and crimps/in. in the 6.35 cm lengths of the selected staples. The test lengths with the shortest and longest mean fibre lengths of 7.37 cm and 8.33 cm were both strong crossbreds with 1.8 and 1.7 crimps/in. respectively. The mean fibre length/test length ratios of these two samples were 1.16 and 1.31, while the mean for all samples was 1.25. The variation in fibre length within a sample was not large, the coefficients of variation of

TABLE 2. Fibre Length Data for Test Length of 6.35 cm

Sample No.	Crimp / in.	Fibre Length (cm)	C.V. (%)	F.L.* / T.L.	% Of Fibres In Cm Length Groups				
					6–7	7–8	8–9	9–10	> 10
9	14.4	8.06	12.3	1.27	5.7	43.8	32.8	12.6	5.1
10	12.6	8.06	10.9	1.27	10.9	35.4	38.9	13.0	1.8
3	7.7	8.11	11.6	1.28	12.0	32.3	39.0	14.6	2.1
11	5.9	8.32	15.3	1.31	15.5	32.4	25.1	14.0	13.0
1	4.9	7.89	12.8	1.24	23.2	36.2	21.9	17.4	1.3
2	4.7	7.92	11.3	1.25	18.8	35.1	33.1	12.7	0.3
4	4.3	7.66	9.5	1.21	11.4	63.1	18.8	6.7	—
5	3.2	7.77	10.7	1.22	14.2	52.8	25.5	5.5	2.0
7	3.1	8.10	12.6	1.28	8.2	43.2	29.3	16.3	3.0
6	2.8	7.92	13.4	1.25	23.6	33.8	23.5	16.9	2.2
8	2.4	7.54	12.6	1.19	30.1	41.5	21.7	4.2	2.5
12	1.8	7.37	10.1	1.16	23.3	61.8	11.0	2.4	1.5
13	1.7	8.33	12.4	1.31	5.1	39.6	27.4	20.8	7.1
Mean	5.3	7.93	12.0	1.25	15.5	42.4	26.8	12.1	3.2

*F.L. = Fibre Length. T.L. = Test Length.

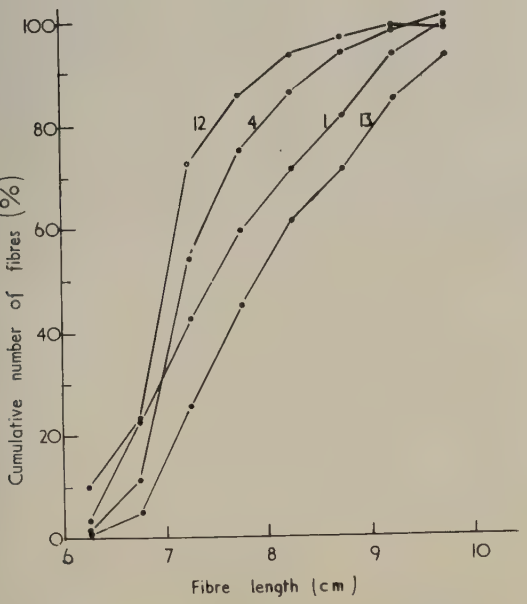

Fig. 2.—Cumulative fibre length distribution of 4 samples with a test length of 2.5 in. (6.35 cm).

fibre length ranging from 9.5% to 15.3%, with a mean of 12.0%. This is similar to the mean coefficient of variation of 11.7% found in Merino staples by Lang and Chaudhri (1948). It may be seen from Table 2 that there were large differences between the percentages of fibres which fell within the different length groups. The fibre length distributions had a skew varying from zero to a small positive value.

Fig. 2 illustrates the length distributions of samples Nos. 1, 4, 12, and 13 which cover the range of the tests. Samples Nos. 12 and 13 had similar crimp and fibre diameter but showed the widest variation in mean fibre length. If breaking stress and fibre extensibility were similar for these two wools, sample No. 12 would reach its maximum load sooner and at a lower extension than sample No. 13. It is also probable that sample No. 12 would sustain a higher maximum load than sample No. 13, as a greater number of fibres would be under tension at one time. The extension at staple break of sample No. 12 would, however, be smaller than that of sample No. 13.

These fibre-length/test-length values indicate that the ratio of 1.06 found by Ross (1959) for 11 samples of Romney Crossbred wool was low; this was due, no doubt, to fibre breakage and shed fibres.

TABLE 3. Mean Fibre Diameter of Cm Length Groups

Sample No.	Mean Diameter μ	Diameter of Cm Length Groups, μ			
		6–7	7–8	8–9	9–10
9	20.1	22.5	18.8	20.5	22.2
10	20.3	18.5	18.7	21.1	22.5
3	22.0	18.3	20.5	22.4	25.7
2	29.6	26.9	27.9	30.6	33.3
1	30.1	27.1	28.7	31.9	34.7
11	30.9	28.3	29.5	32.2	33.2
4	35.2	31.4	33.5	39.1	41.2
6	36.2	29.7	35.6	40.1	37.7
7	37.3	31.7	33.7	39.9	41.4
5	37.7	36.8	36.6	38.8	41.7
12	39.8	38.2	39.1	43.2	44.4
8	40.4	36.5	41.4	41.9	43.7
13	40.7	37.4	38.7	40.7	44.5
Mean	32.3	29.5	31.0	34.0	35.9

Table 3 shows that an increase in fibre length is accompanied by an increase in fibre diameter. The longest fibres, which were also the largest in diameter and consequently would have the highest breaking load, would be the last to extend and break as the staple was loaded. An accurate assessment of the effect of changes in fibre diameter is not possible without knowledge of the relation between breaking load/unit area of fibre cross-section and fibre diameter. The data on this point are conflicting, and no doubt vary

for different wools. Anderson and Cox (1950) and Ross (1960b) reported increases in breaking stress with increasing fibre diameter for Romney wool. Anderson and Cox (1950) found a small positive correlation between extension at fibre break and fibre diameter for fibres from a few Romney lamb staples, the mean breaking extension being 39.4%. If the longest group of fibres (at a test length of 6.35 cm) had a length of 10 cm and a breaking extension of 40%, then the extension at staple break would be 120%, which is of the same magnitude as was found for pre-and post-lamb shorn staples (Ross 1960a).

The topic of follicle competition for fibre substrate, particularly in times of stress, is of considerable interest. As mentioned in Part I of this series (Ross 1960a), a new approach may be made to this subject from the study of staple breaking graphs. A detailed analysis of such graphs is, however, not possible, as it would require a knowledge of fibre length, diameter, extensibility, breaking load, and breaking stress for each individual fibre in the staple. Nevertheless, the principal factor determining the shape of the breaking graph will be the relation between fibre length and breaking load. As the longest fibres are the largest in diameter they will, in general, be of primary follicle origin, while the shorter finer fibres will have been produced by secondary follicles. Fig. 3 illustrates various types of breaking graphs which were found when testing Romney post-lamb shorn staples at a test length of 2.5 in. (Ross 1960a).

Graphs A, B, and C are of sound staples. Type A, the most common, will no doubt result from a normal fibre length distribution of a sound staple. There is a distinct "tail" region in which the longest and coarsest fibres break. Graph B illustrates a sound staple in which there is very little variation in fibre length. This results in a high maximum load and a low extension at staple break. The longer extension at maximum load of graph C may be due to several factors: few short fibres; or short fibres which are either extensible or very week, resulting in a long staple-"uncrimping" region.

Graphs D, E, F, G, H, and I illustrate staples of medium strength, which have a lower maximum load than sound staples. If the fibre length distribution is similar to that of sound staples then the short fibres have been progressively less affected by the break in the series D, E, F, and G. In G the fibres of medium length are not particularly strong, while in H they are quite weak. In graph I it is the short fibres which are sound. Graphs of the type D and E occur most frequently, although type I graphs are not uncommon. Only one distinctly two-peaked graph, H, with a slight decrease in strength between the peaks, has been found in relatively sound wool, although with weaker staples two-peaked graphs with a slight decrease in strength between the peaks are not uncommon.

Graphs J, K, L, and M are of very weak staples, and they will result if very thin staples of medium soundness are tested. The most common type is L, in which the load increases slowly, reaching a maximum just prior to staple break. Types K and M, in which

there is a distinct maximum load peak due to the long and the short fibres respectively, are also found.

It should be remembered, in interpreting these breaking graphs, that there is a high positive correlation between maximum load lb/g and work cm lb/g, so that, in practice, graphs A to C will be of a considerably greater area than graphs D to I for staples of a similar weight.

If a decrease in staple strength results in a change in the fibre length distribution, then graphs such as D to I might be interpreted in terms of fibre length distribution alone. In D there would be

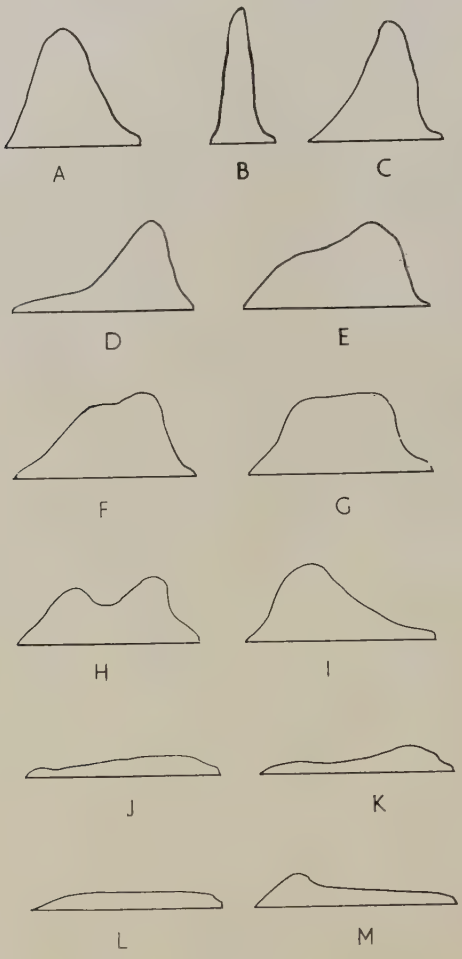

Fig. 3.—Breaking graphs of staples of varying degrees of soundness.

few short fibres, in I a large proportion of short fibres, while for H there would be a group of short and a group of long fibres. No doubt the factors which cause a break affect both the fibre strength and the fibre length distribution in the staple. This is shown by the decrease in the work required to break the weaker staples, and the differences and variability of their breaking graphs, compared with sound staples. The differences in the breaking graphs of weak staples, however, may be attributed principally to changes in breaking stress, rather than to changes in fibre length distribution.

Variation in Staple Strength over the Fleece

General

It is apparent when working with fleeces that there may be marked differences in the degree of break from one region of the fleece to another. Such variations must result from differences in the response of the wool follicles in different skin regions to periods of stress, superimposed upon the normal gradients in staple strength in "stress-free" sheep. The strength of wool fibres may also be markedly reduced by weathering, although, as this is largely confined to the tips of the staples, it would not be expected to affect the lambing break region. A large proportion of Romney ewes, however, have open mushy backs in which the staples appear to be weathered almost down to the skin level. In this instance weathering might be expected to reduce the minimum staple strength. Data on the variation in staple strength over the fleece are desirable from the point of view of both sampling and the interpretation of staple strength tests, as well as from the more fundamental aspects of basic studies on wool growth over the body of the sheep.

Experimental Procedure

The staple strength was measured at a test length of 2.5 in. on two or more full-year staples from each of six positions on three groups of post-lamb shorn Romney ewes. The sampling positions used were:

(1) Side: the mid-side position over the last rib.

(2) Back: on the dorsal line immediately above position No. 1.

(3) Shoulder: midway between the point of the elbow and the back of the leg.

(4) Withers: on the dorsal line immediately above position No. 3.

(5) Neck: midway between the point of the shoulder and the ear.

(6) Britch: midway between the stifle joint and the back of the leg.

Results and Discussion

Table 4 shows the mean values for maximum load, work, extension at maximum load, and extension at staple break, for each

group of samples. Analysis of these results showed highly significant differences among both sheep and positions, with the exception of the extension at staple break, in which case only the differences between sheep for the withers samples were significant.

TABLE 4. VARIATION IN STAPLE STRENGTH OVER THE FLEECE (TEST LENGTH 2.5 IN.)

Group	Position	Maximum Load (lb/g)	Work (cm lb/g)	Extension at Maximum Load (%)	Extension at Staple Break (%)
10 Ewes 1955	Neck	101.5	369	41	98
	Shoulder	85.0	308	47	99
	Britch	77.8	267	42	96
	Withers	76.3	320	42	102
	Side	70.7	268	59	106
	Back	58.4	255	53	102
10 Ewes 1956	Neck	94.5	424	49	110
	Shoulder	84.3	363	53	110
	Britch	79.8	335	54	110
	Withers	79.0	378	46	128
	Side	72.8	349	62	117
	Back	75.1	322	53	109
37 Ewes 1958	Neck	53.4	177	43	101
	Shoulder	34.6	108	41	91
	Britch	34.9	106	40	93
	Withers	31.5	110	42	96
	Side	32.9	133	50	112
	Back	27.6	93	41	91

In general, there is a reduction in maximum load through the series neck, shoulder, britch, withers, side, and back. In each year the neck samples were the strongest; and, in the 1958 samples, which had a very severe break, the samples from the neck were very much stronger than those from any other position.

A measure of the degree of weathering or fibre degradation may be obtained by measuring the alkali solubility of the wool,

TABLE 5. MEAN STAPLE STRENGTH AND ALKALI SOLUBILITY DATA FOR 5 OF THE 1958 EWES

Sample	Maximum Load (lb/g)	Work (cm lb/g)	Alkali Solubility (%)
Neck	32	126	8.4
Shoulder	33	118	8.5
Britch	28	87	9.7
Side	26	109	10.5
Withers	18	72	17.5
Back	18	69	20.9

i.e., the percentage loss in weight on an oven-dry basis of samples treated with 0.1 *N* sodium hydroxide for 1 hour at 65°c (Smith and Harris 1936). In this test the greater the degree of fibre damage in a sample the higher will be its alkali solubility.

The results of tests of the alkali solubility of samples from the six positions of five of the 1958 ewes, together with the corresponding mean maximum load and work results, are given in Table 5.

It may be seen that the soundest wools of the neck and shoulder had the lowest alkali solubility and the very weak back and withers samples had very high alkali solubilities. This may account in part for the low strengths of the back samples in 1955 and 1958, although the withers samples, which were almost as weathered, were as strong as the side samples, in which weathering, as indicated by alkali solubility, was not high. As the amount of skin movement in the withers region would be high, any free fibre ends might felt, and perhaps result in a high strength value. Deductions on staple strength from alkali solubilities, however, may be misleading due to the fact that one is in most instances a measure of tip damage, while the other is a measure of fibre strengths at their minimum diameters which are usually near the butt end of the staples. The mean maximum loads for the ewes were: 46, 31, 25, 18, and 11 lb/g, while the corresponding alkali solubilities were 11.2%, 11.6% 14.8%, 12.6%, and 12.7% respectively. The large differences in mean staple strength between the sheep were not reflected in their mean alkali solubilities.

In each year the side samples were much more extensible at maximum load than samples from the other five positions, while, in two years, they also extended farthest at staple break. The similarity of the extensions at staple break for all positions provides further evidence that the longer coarser fibres are least affected by the factors causing a break (Ross 1960a). The variations in extension, particularly at maximum load, indicate differences in the relation of fibre length to test length over the fleeces, and/or differences in the extensibility and composition of the fibres from different sampling positions.

With the exception of the back wool there were only small differences in staple length between the various positions. The diameter of the fibre was similar for the neck and shoulder samples; 3–4 microns coarser for the side, withers, and back samples; and 4–5 microns coarser for the britch samples.

These results indicate differences in the availability of fibre substrate to the follicles in different body regions in times of stress. They do not support the theory that wool growth, at least in strength, is most vigorous in those body regions which are closest to the "vital organs". However, data are required on the staple strengths over the body on wool grown before any stresses due to pregnancy or winter feed shortage occur.

The use of rugs to avoid the effects of weathering might alter the relative staple strengths of the different positions, for although

the break region is close to the butt, the back and withers wool of many Romneys appears to be weathered right down to the skin level.

A sample taken from the normal mid-side sampling position would give a measure of the general staple strength over the body of the ewe, while a neck sample would give a measure of the fleece region which was least affected by the factors causing the break.

Variation between Staples within Samples

As a result of the wide variation in staple strength over the fleece, the variation in strength between the staples of one sample will increase with increasing sample size. As well as these gradients in staple strength over the body of the sheep there is further variation owing to both the method of testing employed and the mechanics of the strength tester. The method of test may be altered depending on the information required, the type of wool, and the general level of strength of the samples. As pendulum strength testers are not accurate over the lower 10% of the scale, it is necessary to select larger staples of weak wools to avoid this region, or to avoid very weak staples being broken by the weight of the clamps alone. As discussed in Part I of this series (Ross 1960a), even in sound wools it is desirable to keep the size of the staples between the clamps as uniform as possible, sample size having an appreciable effect on both maximum load and work even though these are expressed in lb/g of wool cut from the clamps after breaking. Sample size also affects extension at maximum load and staple break.

The following coefficients of variation between staples within samples apply to samples of Romney wool, at a test length of 2.5 in.

Ten staples were tested from each of 10, 100–200 g wool samples, which covered the normal range of staple strengths. The clean scoured weights of the broken sections of the staples for any one sample varied over a range of approximately 1 to 2. The coefficients of variation of maximum load varied for these different samples from 5% to as high at 60%, with a mean of 16.1%, the variation being higher in the weaker samples.

Two staples per sample were tested from the 222 weak samples (50–100 g) from 37 ewes (Table 4). The between-staple coefficients of variation for maximum load lb/g, work cm lb/g, extension at maximum load, and extension at break were 20.2%, 21.4%, 17.4%, and 6.5% respectively. Corresponding values for a group of 20 sound samples, testing 10 staples per sample, and keeping the weights of the staples of each sample to a range of approximately 1 to 1.5, were 10.2%, 8.4%, 5.0%, and 14.3%. With these sound staples the variation within samples was much lower for maximum load, and work and extension at maximum load, but higher for extension at staple break. The respective values for the sample with the least variation were, 5.1%, 5.0%, 3.2%, and 7.1%.

With several other groups of wool of medium to low strength, coefficients of variation of maximum load varied from 14% to 22%. Depending on the strength of the wool to be tested, a coefficient of variation of from 10% to 20% should give a satisfactory basis for the calculation of the number of staples per sample which need to be tested to obtain any desired degree of accuracy.

Acknowledgment

The author is indebted to Dr L. F. Story, Director, New Zealand Wool Industries Research Institute, for helpful advice and criticism.

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THE TOXIC AND ANTIGENIC COMPONENTS OF *Sporidesmium bakeri*, Syd. (Syn. *Pithomyces chartarum* (Berk. & Curt.) M. B. Ellis)

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(Received 10 September 1960)

Summary

Crude aqueous spore extracts of the fungus *Sporidesmium bakeri*, Syd. (Syn. *Pithomyces chartarum* (Berk. & Curt.) M. B. Ellis) have been studied by electrophoresis and gel diffusion methods. The toxicity of such material for tissue culture cells has been confirmed and a modified assay procedure developed.

Unsuccessful attempts have been made to demonstrate *in vitro* toxin neutralising antibodies in the sera of guinea-pigs dosed with *S. bakeri* spores, rabbits hyperimmunised with fungal material, and a small number of sheep suffering from facial eczema.

Introduction

Hepatic lesions similar to those exhibited by sheep suffering from facial eczema have been produced in guinea-pigs, rabbits, and lambs by administering the fungus *Sporidesmium bakeri*, Syd. (Syn. *Pithomyces chartarum* (Berk. & Curt.) M. B. Ellis) either as a crude suspension or as the chemically-defined compound sporidesmin (Clare 1959; Evans *et al.* 1957; Perrin 1957; Synge and White 1959; Thornton and Percival 1959). The macroscopic appearance of such lesions, particularly in young guinea-pigs where a direct dose-response relation followed the feeding of grass or fungal cultures, has formed the basis of a toxicity assay (Perrin 1957). This procedure has recently been modified by MacKinnon and Te Punga (1961) to include direct oral dosing of test material and the microscopic grading of liver lesions according to their involvement in vascular and biliary systems. Nevertheless, the method remains both laborious and time-consuming. The finding by Murphy and Worker (1960) that a close relation existed between guinea-pig assay results and the ability of toxic extracts to produce cytopathic changes in tissue cells suggested possible application of this latter technique in further studies on facial eczema. To this end, the present project was undertaken to confirm Murphy and Worker's observations with a view to their subsequent use in a serological study of the fungus components in general and of the hepatotoxin in particular.

Experimental Procedure

Fungal Material

Dr R. H. Thornton kindly provided several batches of spores obtained from 21-day wheat grain cultures of *S. bakeri*. The detergent-water harvesting fluid was removed by suction and the air-dried spore cake stored at —20°c. For guinea-pig dosing experiments a stock 1% w/v suspension in distilled water was prepared daily.

Spore extracts were obtained by grinding spores in a Moorehouse mill using phosphate-buffered distilled water (0.001M, pH 7.2) as diluent. A 50% w/v suspension of 1.5 kg spores was recycled four times between grinding discs set approximately 10μ apart. The resulting extract containing few intact spores was stored at 4°c for three days and then centrifuged. The supernatant was kept cold while the cell debris was re-extracted for three days with twice its volume of buffered water. After centrifuging, the two cell-free extracts were combined and either deep-frozen in small volumes or lyophilised in such quantity as to permit a 20-fold concentration upon reconstitution.

Specimens of purified sporidesmin were obtained from Dr E. P. White, Ruakura Animal Research Station.

Tissue Cultures

Four continuous cell lines were employed. Cultures of HeLa and pig kidney were provided by Dr J. Done, Ruakura Animal Research Station, while human foreskin (FS) and rabbit spleen cells, together with a further HeLa culture, were kindly provided by Dr W. Hamilton of the National Health Institute, Wellington. These cells, together with primary cultures derived from a number of animal species, were prepared by conventional trypsinisation procedures and maintained in a medium composed of Hanks' solution (75 parts), unheated calf serum (10 parts), 5% lactalbumin hydrolysate (10 parts), 5% yeast extract (5 parts), and containing the following antibiotic concentrations per ml: penicillin—100 units; streptomycin—50 μg; and mycostatin—20 units (Melnick 1956).

Toxicity Assays in Tissue Culture

The observations of Murphy and Worker (1960) were readily confirmed. Aqueous extracts of spore material in dilutions of 1:10–1:250 produced rapid alteration in morphology of HeLa cell cultures and ultimate disintegration of the cell sheet. To determine the particular tissue most suitable for assay purposes, semi-confluent monolayers of cells from different animal species were produced and the culture fluids replaced with 1 ml of doubling dilutions of 50% milled spore extract prepared in nutrient medium. Following incubation at 37°c for four days, the highest dilution of extract which resulted in contraction and spindle formation of individual cells (as distinct from the contiguous cell sheets of control cultures) was considered to contain 1 Tissue Culture Dose (TCD) of toxin per ml.

As shown in Table 1, considerable variation in cell susceptibility was evident. While none of the cultures tested was entirely refractory to toxin, both primary and continuous cells of rabbit origin showed only minor changes after four days' incubation in spore extracts suffi-

TABLE 1. COMPARATIVE SENSITIVITIES OF CELL TYPES TO *S. bakeri* EXTRACT

Cell Type	Culture History	Toxicity
HeLa	Continuous	1,500*
FS	Continuous	3,000
Rabbit spleen	Continuous	50
Rabbit kidney	Primary	10
Dog kidney	Primary	1,500
Pig kidney	Continuous	1,000
Pig kidney	Primary	500

* Reciprocal of extract dilution containing 1 TCD toxin per ml.

ciently toxic to cause the sloughing off from the tube walls of other cells within 18 hours. With pre-formed cell sheets, the end-point was by no means reproducible and could vary by as much as a four-fold dilution when titrations were conducted with different cell concentrations. Using the most sensitive tissue system, the FS cell line, attempts

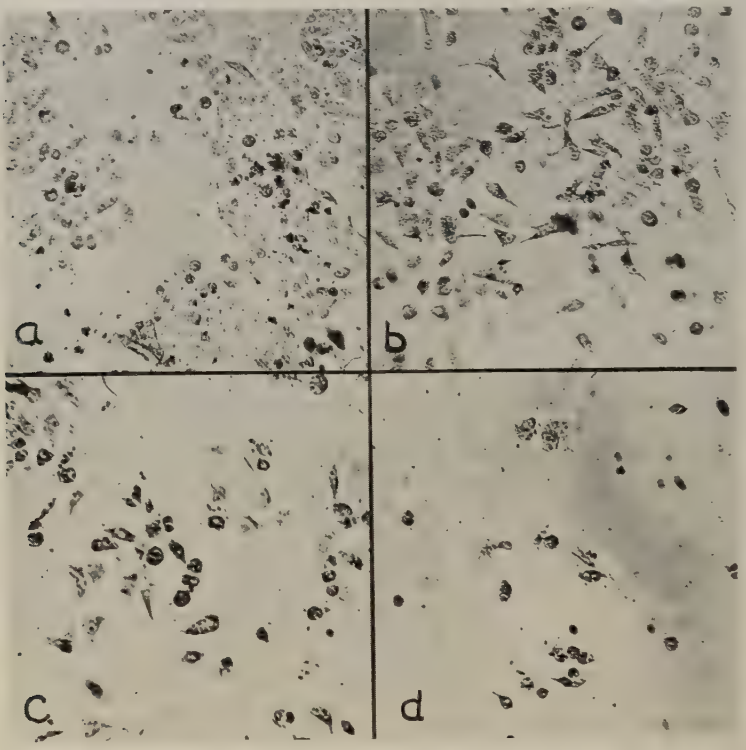

Fig. 1.—Cytopathic effects of *S. bakeri* toxin on FS cells. Cultures incubated 4 days at 37°c (unstained, x 175). Figures refer to dilutions of 50% aqueous spore extract: (a) nil; (b) 1:4,000; (c) 1:1,000; (d) 1:250. Titration end-point = 1:4,000 dilution.

were made to increase the sensitivity of the assay procedure. When trypsinised cell suspensions were incubated together with dilutions of toxin a direct relationship appeared to exist between the number of cells and the end-point of a given toxin preparation. This observation formed the basis of the following assay procedure. To two-fold dilutions of toxin (0.5 ml) in nutrient medium was added an equal volume of trypsinised FS cell suspension standardised by direct counting to contain 175,000–200,000 per ml of medium. The tubes were tightly stoppered, shaken, and incubated in the sloping position at 37°c for four days. The results were reproducible with different batches of test cells and the end-points well-defined (Fig. 1).

Neutralisation Tests

Attempts to detect tissue culture neutralising antibodies in different sera were made using a nine-tube "chess-board" pattern for each serum sample—0.25 ml serum dilutions (undiluted, 1:5, 1:50) and 0.25 ml aqueous spore extract dilutions containing 100, 20, and 8 TCD toxin. The mixtures were held at room temperature for two hours, and to each tube was then added an equal volume (0.5 ml) of standardised FS cell suspension. The tubes were sloped and incubated at 37°c for four days or until suitable controls on toxin and cells gave the anticipated readings.

Antisera to S. bakeri

Hyperimmune sera were prepared by injecting three groups of rabbits with 20 times concentrated aqueous spore extract combined with an equal volume of saline, aluminium hydroxide gel, or Bayol F-Arlacel A adjuvant. The initial inoculations (0.75 ml) were given by the deep subcutaneous route. Ten days later all animals commenced receiving a bi-weekly series of intraperitoneal injections of 6 ml unconcentrated spore extract. After four weeks the rabbits were rested 10 days and bled by cardiac puncture. The sera were stored deep-frozen in the unheated state. A similar immunising schedule was commenced in guinea-pigs, but deaths from anaphylactic shock, toxicity, or intercurrent infection prevented the accumulation of representative serum stocks.

Blood taken from sheep and guinea-pigs following their exposure to toxic pasture or direct dosing with *S. bakeri* spores respectively was also included.

Results

Attempts were made to characterise the aqueous extracts of *S. bakeri* spores by applying electrophoresis, gel diffusion, and immunoelectrophoresis procedures.

Electrophoresis Studies

An EEL (Evans Electroselenium, Limited) power unit and bath assembly was used. Samples of 0.02 ml were placed on Whatman No. 1 paper strips 5 cm in width and a current of 0.4 ma/cm applied for periods of 8–24 hours. The bath contained veronal buffer, pH 8.6, of ionic strength 0.075. Following electrophoresis, the paper strips were dried at 37°c, halved longitudinally, and one half sprayed with

the iodine-azide reagent for sulphur-containing amino acids as described by Russell (1960), for the detection of sporidesmin. The ninhydrin reaction for amino acids and peptides was performed on the duplicate strip. Separate preparations were stained by the bromophenol blue reagent for protein, as were strips containing dog serum simultaneously analysed by electrophoresis for reference purposes.

Separations were performed upon an aqueous spore extract concentrated 20 times, purified sporidesmin, and dog serum. In addition to the staining procedures described, toxicity for tissue cultures was determined by cutting the paper strip transversely into 2-cm panels and eluting each into 0.5 ml nutrient medium. This was added in 1-ml volumes to FS cell cultures at a further dilution of 1 in 10.

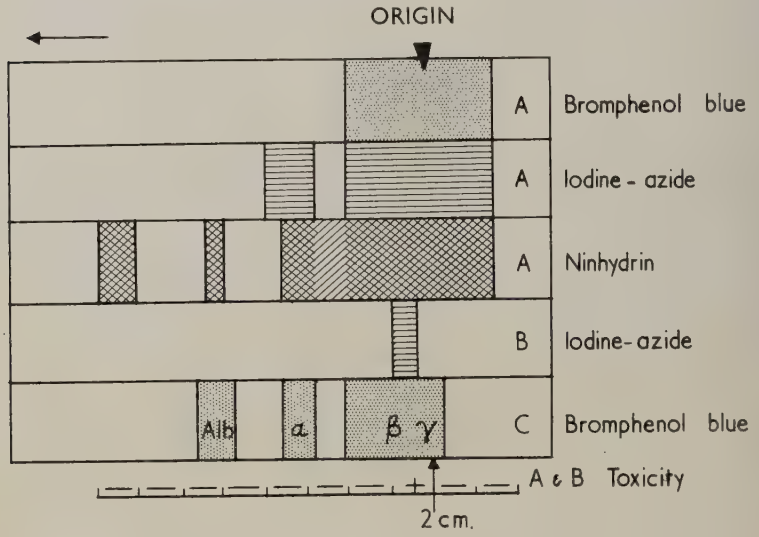

Fig. 2.—Paper electrophoresis of concentrated aqueous spore extract of *S. bakeri* (A); purified sporidesmin (B); dog serum (C).

Fig. 2 indicates that the sample of sporidesmin (B) migrated slowly during an 18-hour period, showing a single zone of iodine-azide reacting material which corresponded to the panel possessing tissue culture toxicity. On the other hand, toxicity was restricted to a relatively small portion of the two iodine-azide reacting areas produced by crude spore material (A). Staining with ninhydrin revealed the presence of well-defined mauve bands, two corresponding to the iodine-azide areas, a third 10 cm beyond the point of origin, and a particularly fast-moving zone at the extreme anodal edge of the strip.

Electrophoresis of spore extracts previously heated at 100°c, while showing little alteration in iodine-azide patterns, exhibited toxicity reductions from 5,000 TCD per ml of unheated material to 1,000 TCD (30 minutes), 500 (60 minutes), 100 (90 minutes), and 75 (two hours). The four mauve ninhydrin bands remained constant, with a

marked increase in inter-zonal coloration—a pale blue panel 4 cm from the origin—appearing in specimens heated for 30 minutes or more.

Serological Studies

Two procedures were employed: the tissue culture toxin neutralisation test described earlier; and the Ouchterlony gel diffusion reaction. In the latter case agar gels 2 mm in depth were formed in Petri dishes and four holes 5 mm in diameter cut equidistantly about a central well measuring 7 mm in diameter. The medium consisted of 1% Difco agar in distilled water, pH 7.2, containing 0.2% phenol. The definition of precipitin bands was enhanced by the addition of 0.75 ml of 0.3% methyl orange solution. For the screening of many sera a single antigen sample was contained within the central well while the antisera under test occupied the surrounding holes. Precipitin bands were observed by oblique illumination after 48 hours' incubation at 37°c.

In addition to sera obtained from rabbits hyperimmunised with spore extracts, 13 sheep from long-standing cases of facial eczema, and 14 from lambs exposed four weeks previously to toxic pasture, were available for examination. A number of individuals, as well as pooled guinea-pig sera from spore-dosing trials performed in association with Miss Barbara Smith, were also included. Two of these latter experiments are summarised to provide a background for the serological studies presented in this section. In Experiment 2, groups of 120 g guinea-pigs received orally 25, 50, 150, and 250 mg intact spores distributed over a dosing period of five days. Fourteen days later the animals were bled and autopsied. Liver sections from the two highest dose rates showed moderate facial eczema lesions (Grade 2–3 in MacKinnon and Te Punga's classification). Under similar conditions 450–500 g guinea-pigs received 150, 250, 350, 500, 750, and 1,000 mg spore material. The histological findings, even in animals receiving the upper dose range, were confined to small areas of bile ductule proliferation irregularly distributed throughout the parenchymal tissue.

Experiment 4 represented an attempt to immunise guinea-pigs with crude toxin—adjuvant mixtures. Here, sera were collected from groups of animals 30 days after having received subcutaneously 0.75 ml of 20 times concentrated aqueous spore extract mixed with an equal volume of saline, aluminium hydroxide gel, or Bayol F-Arlacel A oil. The immune status of each animal, together with that of unvaccinated controls, was then determined by giving orally a total of 1.5 g of *S. bakeri* spores over a six-day period. All animals were killed after a further 14 days. The histological findings showed that no resistance was conferred by any of the mixtures used for vaccination: indeed, there was evidence to suggest that animals which had received toxin in combination with oil or alum adjuvants prior to spore challenge possessed more advanced lesions (Grade 4) than those with toxin and saline or spores alone (Grade 2–3).

With all the above sera, attempts to demonstrate the presence of antibodies capable of protecting FS cells against toxin were unsuccessful. This was the finding irrespective of whether a saline spore extract or 0.002 μg sporidesmin (both equivalent to 2 TCD) was the test agent. Even with sera from rabbits hyperimmunised with fungal spore extracts

where, allowing for dilution factors, the lowest serum dilution tested was 1:4, the cytopathic effect of this minimal dose of toxin was still manifest. Incubation of the serum-toxin mixtures was prolonged to 18 hours at room temperature, eight hours at 37°c, or 48 hours at 4°c, without affecting this result. It was concluded that, under the test conditions described above, antibodies capable of neutralising the toxic effect of *S. bakeri* extracts could not be detected.

The same sera were examined for their ability to form visible precipitates in agar gels using concentrated spore extracts as antigen. All sera from sheep and guinea-pigs in Experiment 2 failed to react. With the exception of vague opacities produced by an occasional serum from animals which had received toxin-oil mixtures (Experiment 4), the remainder were likewise negative.

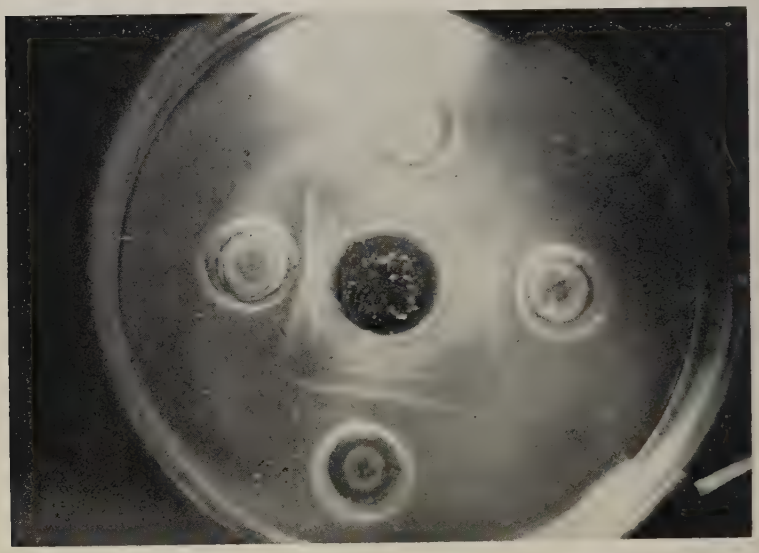

Fig. 3.—Gel diffusion test showing effect of distance on precipitation bands. Concentrated spore extract in outer wells, homologous rabbit antiserum in central well.

More success followed the use of sera from rabbits hyperimmunised with aqueous spore extracts. Here a series of five well-defined precipitation bands were formed against the homologous antigen (Fig. 3). When this crude material was subjected to immunoelectrophoresis by the method of Williams and Grabar (1955) at least four antigens possessing different mobilities could be resolved (Fig. 4a). Optimal conditions for separation using a plate size of 17.5 × 13 cm were provided by a pH of 8.6 and a current of 12 ma for 18 hours at room temperature. Exposure of the crude spore extracted to 100°c for 30 minutes resulted in elimination of the two upper components, while a third was removed after a further 30 minutes' heating (Fig. 4b). An interesting finding was the appear-

ance, in extracts heated for one hour or more, of a new zone of precipitation apparently unrelated to any component existing in the original material (line x, Figs. 4a and b). It will be recalled that paper electrophoresis of spore extracts heated under similar conditions showed no alteration in iodine-azide-reacting components over a 90-minute period and that toxicity for tissue cultures was reduced to one-fifth of the original level after 30 minutes and to one-tenth after one hour at 100°c.

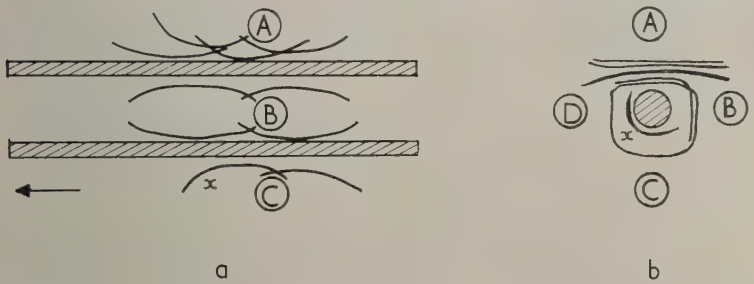

Fig. 4.—(a) Immuno-electrophoresis of concentrated aqueous spore extract. Unheated (A); 100°c for 30 minutes (B); 100°c for 60 minutes (C). Rectangular troughs contain *S. bakeri* rabbit antiserum.

(b) Gel diffusion tests of material in Fig. 4a. Extract heated at 100°c for 2 hours (D). Central well contains antiserum.

All attempts to obtain specific precipitation between a sample of purified sporidesmin and hyperimmune rabbit serum were unsuccessful. No reaction appeared in systems which contained the highest concentration of sporidesmin tested (80 μg) and concentrated globulins from such sera.

Zone electrophoresis was used to characterise the material present in concentrated aqueous spore extracts. Employing a cellulose powder slurry made in veronal buffer of pH 8.6, and contained within a Perspex trough (dimensions 23.5 × 3.2 × 0.3 cm), samples of 0.1 ml volume were analysed by electrophoresis at 12 ma. During a period of 24 hours the bed was "printed-off" on chromatography paper for iodine-azide staining to determine the degree of separation. This appeared to be optimum after 18 hours. Commencing on either side of the sample introduction slot, the bed was divided transversely into a series of 2-cm panels, each being subsequently eluted into 1 ml of saline for two hours at 4°c. All eluates were then examined for their toxicity to FS cells; their antigenic composition, using the gel diffusion test; and their reactions to ninhydrin and iodine-azide reagents.

Three coloured zones occupying positions shown in Fig. 5, and corresponding to deep mauve ninhydrin-positive areas, were present in the post-electrophoresis slab. As expected, toxin was confined to a strip included within the iodine-azide panel; these two components were separated from the antigenic material by virtue of the more rapid mobility of the latter.

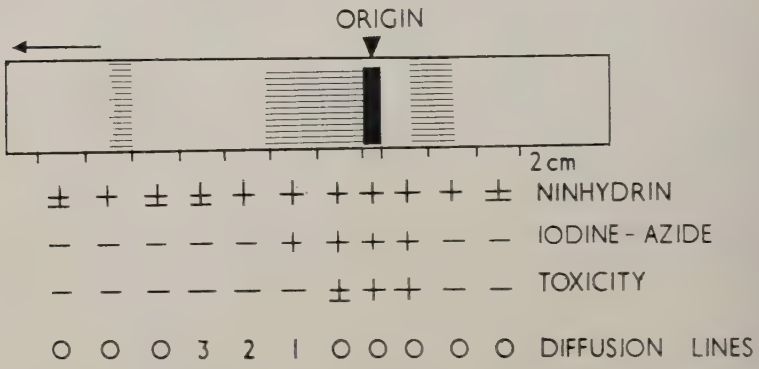

Fig. 5.—Cellulose zone electrophoresis of concentrated aqueous spore extract. Elution panels at 2-cm. intervals.

The above results suggest that the facial eczema toxin is distinct from antigens responsible for the major gel diffusion precipitation reactions. Further, these findings confirm the data obtained from paper electrophoresis analyses that, in crude spore extracts, only a small proportion of the iodine-azide reacting components possess toxic activity.

Discussion

The present investigation may conveniently be discussed in two sections: the first concerns a modification of the tissue culture procedure for assaying the toxin of *Sporidesmium bakeri*; the second considers its application, together with other *in vitro* techniques, to an immunological study of this fungus.

In tissue culture titrations of virus activity, continued virus growth permits cytopathic effects to be interpreted on an "all-or-none" basis; that is, given sufficient time, the culture will become progressively more involved until few morphologically normal cells remain. Substitution of a non-replicating toxin for the viral component alters this system considerably for, unless excess toxin is present, cytopathic changes in pre-formed cell sheets never progress to completion. Furthermore, these changes may become masked by overgrowth from surviving cells. If gross cell death is accepted as an end-point of toxin activity then this figure will invariably be an underestimate of the toxic titre. Its acceptance as a basis for standardising the challenge dose of toxin for any serum neutralisation test would therefore result in a large excess of toxin being present in the mixture, leading to a corresponding loss in sensitivity of the test. Since a major part of the current project was an attempt to detect facial eczema toxin-neutralising antibodies in a variety of sera, it was apparent from the outset that such disadvantages could limit the usefulness of this procedure. An appreciation of these factors resulted in the modified titration method described in the text.

Contained within an aqueous extract of *S. bakeri* spores are compounds which give intensive coloration with ninhydrin, rapidly decolor-

ise the iodine-azide reagent for sulphur-containing amino acids, are toxic for cells in tissue culture, and precipitate in the presence of homologous antibodies. By electrophoresis on both paper and cellulose powder, sufficient separation can be achieved to indicate that the toxic fraction is distinct from the antigenic components responsible for the major precipitation lines in Ouchterlony gel diffusion tests. Furthermore, toxin distribution is restricted to a relatively small portion of the more mobile iodine-azide reacting areas. In comparison with the reference standard of dog serum, the toxin was found to possess a mobility comparable to that of β-globulin, while the iodine-azide complex corresponded to that of the combined globulin fractions. Of the four distinctly coloured ninhydrin-reacting areas, the anodal migration rate of one exceeded that of serum albumin.

Differences in sensitivity to heat have provided additional information about the separate natures of water-soluble spore extract constituents. At 100°c, hydrolysis of both proteins and peptides was indicated by increased inter-zonal coloration with the ninhydrin reagent, a distinctive pale blue band appearing in electrophoresed preparations of extracts heated for 30 minutes or longer. While toxin became progressively weaker—90% activity being lost after 60 minutes at this temperature—there was no concomitant alteration in the distribution or intensity of the iodine-azide staining complex. An interesting observation was the appearance in heated spore extracts of a new antigen apparently unrelated to any present in the original material. This could be readily identified by gel diffusion tests and separated by immuno-electrophoresis. A somewhat similar finding has been recorded for heated pollen extracts by Augustin (1959).

Attempts to demonstrate the presence of precipitins and antitoxic antibodies in the sera of a number of guinea-pigs dosed with spores or extracts of *S. bakeri*, and in the sera from sheep with facial eczema, have been unsuccessful. It could be argued, in view of the negative gel diffusion reactions with purified sporidesmin and hyperimmune fungal antisera, that these results were not unpredictable. Such tests were performed, however, to eliminate the possibility that the purified material may have become degraded during its preparation and no longer be antigenically related to any hypothetical toxin-protein conjugate present in the original spore extract. A second reason for the use of crude spore material was to ascertain whether, in the absence of antitoxic antibodies, the presence of antibodies against other fungal components could be demonstrated. The inability to detect either with the present test procedures suggests that, as in the case of *Aspergillus fumigatus*, immunity is difficult both to detect and to engender (Henrici 1939). This latter point is emphasised by the recent work on production of antibodies to penicillin which required a protracted animal immunisation schedule using adjuvant-antibiotic mixtures (Josephson 1960). An important finding, not without possible parallel in the case of purified sporidesmin, was the additional observation by Josephson that the relatively low molecular weight hydrolytic product of penicillin, penicilloic acid, was incapable of provoking antibody formation. The present work with *S. bakeri* has shown that precipitating antibodies against at least five antigens present in aqueous

spore extracts could be prepared in rabbits. None of these antisera, however, reacted with a sample of purified sporidesmin, and, since supplies of this material were insufficient to attempt antiserum production, confirmation of the lack of antigenicity of the purified hepatotoxin must await further work.

The frequency with which anaphylactic shock was observed in animals repeatedly injected with crude spore extracts suggested the development of sensitivity to some fungal component. A similar inference could be drawn from an unconfirmed observation that liver lesions in guinea-pigs which had received toxin-adjuvant mixtures one month prior to oral challenge with spore material were more severe than those seen in spore-dosed control animals. Furthermore, numbers of eosinophils, cells often indicative of an allergic reaction, were found to be associated with lesions in the former groups. What part any allergic condition plays in the pathogenesis of facial eczema is uncertain. However, it is conceivable that sensitisation to fungal components may predispose an animal to the hepatotoxin, or, alternatively, potentiate the effects of this latter substance.

Acknowledgments

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ROW TRIALS OF GRASSES AT MOLESWORTH STATION

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Summary

A report is given of the performance of some 40 species of introduced grasses grown in rows in a nursery at 2,900 ft altitude in Marlborough, New Zealand, during trials extending over 10 years. Under the described conditions and for the strains used, the more attractive species included, in alphabetical order: *Agropyron cristatum, A. elongatum, A. intermedium, Bromus inermis,* and *Dactylis woronowii.*

Introduction

In 1943 the Botany Division of the Department of Scientific and Industrial Research, at the request of the Department of Lands and Survey, undertook a long-term project of observation and experiment on the Molesworth and Tarndale blocks of high country in inland Marlborough. The work was planned and personally supervised by the late Dr H. H. Allan, then Director of the Botany Division, who continued to contribute advice and practical help for some years after his retirement at the end of 1948. One of several objectives was "to select species of grasses and legumes useful in renovating the grasslands, to propagate them, and to make trials in different localities". Trials began in 1944, and sowings were made annually. This paper reports on the performance of exotic grasses in nursery rows over the subsequent 10 years. Legumes and indigenous grasses are not reported on here; little reference is made to numerous sowings, most of them unsuccessful broadcasts, in places other than the nursery rows.

From 1953 there was great improvement in the vegetative cover on Molesworth and adjacent tussock grassland. This was associated with higher annual rainfalls (30 in. average for the five years 1953–7) and decrease in rabbit numbers; also, in many places, aerial sowing of cocksfoot (*Dactylis glomerata*) has met with good success. There is therefore waning interest in the unfamiliar and, on the whole, less well-adapted grasses here discussed.

Site of Trials

The nursery, situated in the upper Awatere valley at 2,900 ft above sea level, lies very near to the Molesworth homestead and its

Fig. 1.—Molesworth Station: general view showing nursery with trial rows, February 1953. The climatological station is immediately outside the photograph, to the right. (National Publicity Studios photo.)

climatological station (Fig. 1). Actual monthly rainfall is shown in Fig. 2, the average for the relevant years being 26.4 in. Over the 10½ years, rain-days ranged from 82 to 111 per annum; 16 months each had fewer than five rain-days, 28 months each had more than 10 rain-days, and the remaining 82 months each had five to 10 rain-days, with no very clear-cut seasonal pattern. Mean daily earth-

temperatures at depths of 4 in., 8 in. and 12 in. are recorded in Fig. 3, which also shows that, though there was occasionally as long as three months without a screen frost, seedlings were likely to meet freezing temperatures at ground level in any month of the year and almost daily for long periods.

The few 1944 trials occupied a temporary enclosure. In 1946 a lucerne paddock of about three acres was broken up to form a permanent nursery surrounded by a rabbit-proof fence. The portion used for rows was almost flat, but in a few slight hollows water and snow lay long enough to affect growth. The soil, a light gravelly sandy loam, had some very stony patches.

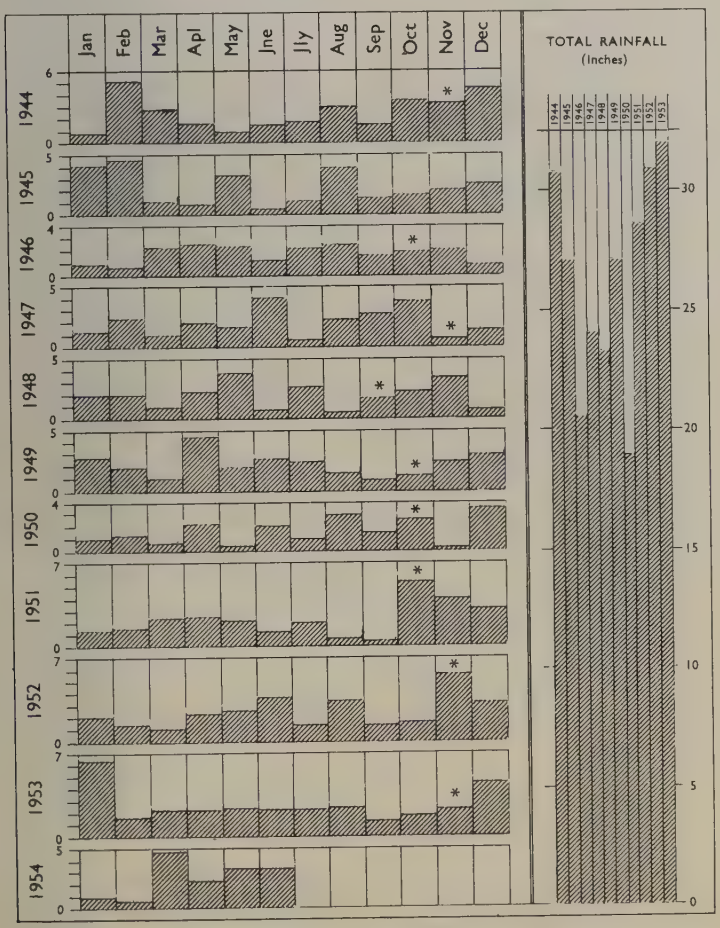

Fig. 2.—Rainfall at Molesworth, in inches. Asterisk (*) indicates month of sowing.

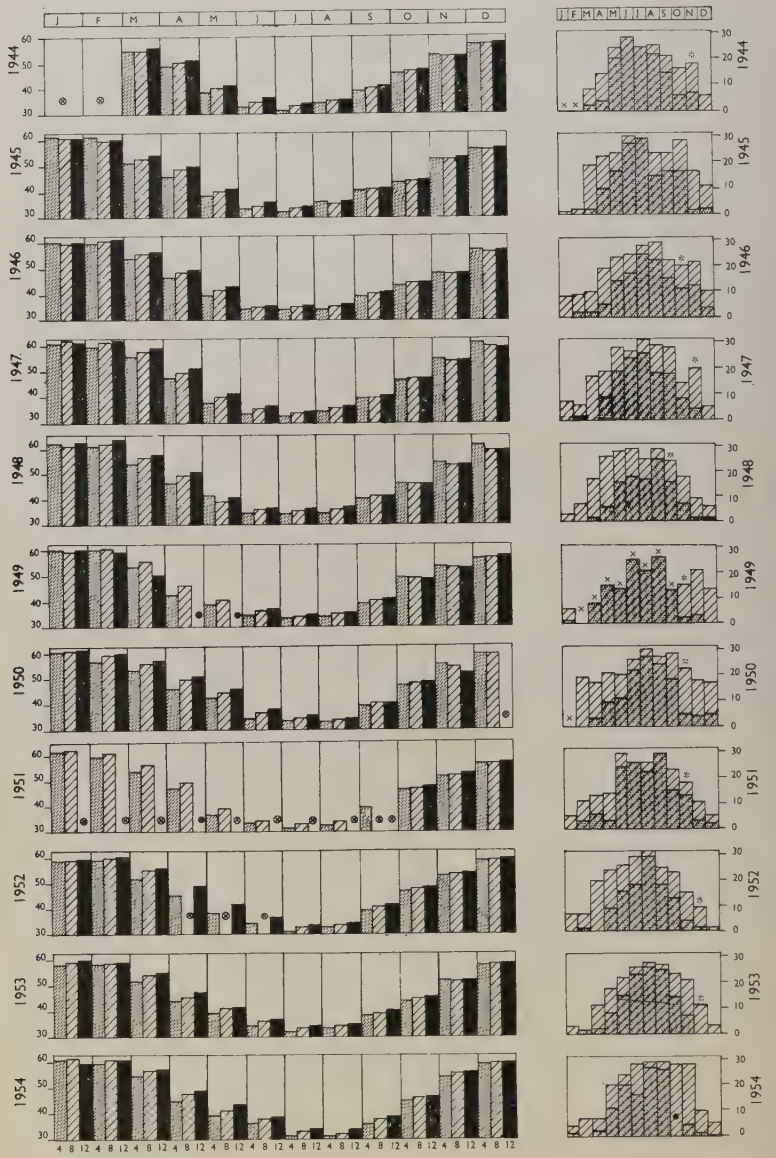

Fig. 3.—Temperatures at Molesworth by months. On left, mean daily earth-temperatures (°F) at 9–9.30 a.m., at depths of 4, 8, and 12 inches; circle indicates no record. On right, numbers of days with screen frosts (stippled) and ground frosts (cross-hatched); circle indicates no record for screen frosts, X no record for ground frosts. Asterisk (*) indicates month of sowing.

Materials and Methods

Seed came mostly from institutions in the United States and Australia, the sources of seed being listed in each case. Names used are those under which the seed arrived, and voucher specimens are held in the Botany Division herbarium. In the first few years imported seed was sown directly at Molesworth; later introductions were first screened at a Divisional nursery at Waiwhetu, Hutt Valley, Wellington, and seed of the more promising accessions was harvested for trial at higher altitudes. Seed from Molesworth rows was tested as it became available.

Rows were up to 70 paces long, the smaller trials occupying only a few yards, the largest as much as two rows. Seeds were sown in furrows about 3 in. deep, at dates varying from September to November (see Fig. 2) depending on when a visit to the area was possible. Cultivation was restricted to ploughing in the spring, hand-grubbing of large weeds, and hoeing down the rows in spring and autumn. Rank growth was occasionally cut back with a mower, and during the last two of the 10 years a rotary hoe was used to advantage. Except for a light initial dressing of superphosphate in 1946, fertilizer was not used.

Weeds were always abundant, the predominant ones being *Poa pratensis, Rumex acetosella, Echium vulgare, Crepis capillaris, Chenopodium album*, and the annual brome grasses that were introduced as impurities with the grass seed. The original lucerne was never fully eliminated, and scattered plants continually reappeared both in and between rows; some can be seen in Fig. 1. Weed competition probably did not in itself cause any species to fail, and in some cases weeds may have been beneficial. Grasses, particularly *Bromus inermis* and *Agropyron intermedium*, grew more vigorously and were much greener near to lucerne plants, and a close cover of weeds noticeably reduced frost-lift.

Trials could be examined usually only twice a year, sometimes as early as September, once as late as May.

No row sowings were made after 1953, and late trials which had then been under observation for only a year or two are not mentioned here. The nursery area was mowed in 1954 and has since been opened intermittently to grazing by small numbers of farm stock of various kinds. Many of the rows were still easily identified in February 1959, and, if the land were not required for other station use, they could continue to provide additional information and be a potential source of propagating material of the more persistent species.*

Results

Results are reported under headings of species, alphabetically arranged; a short supplementary list records some species that did not establish. Brief descriptions (set in 8 pt type), abstracted mainly from the United States Department of Agriculture Yearbook

* By February 1961 the nursery area had been ploughed and resown and carried a good stand of lucerne.

for 1948, provide background information about most of the species and their use and performance in North America.

From spring sowings some species developed only shallow roots by autumn, and then plants were thrown out by frost-heave in the first winter. In late-maturing species seed failed to set except after a long dry summer, as in 1953–4. Amongst the more attractive grasses were *Agropyron cristatum, A. elongatum, A. intermedium, Bromus inermis,* and *Dactylis woronowii,* species all native to the Old World.

Agropyron cristatum

Crested wheatgrass is a hardy, perennial, leafy bunchgrass, well adapted to cool dry areas. It has a wide-spreading, deeply penetrating root system, and can survive cold and drought, withstand grazing, and compete with weeds and associated grasses. Growth begins early in spring, ceases during long dry hot periods of summer, but recommences when moist cool weather returns. Consistently good seed crops can be expected, except when limited by drought. It is long-lived, and sometimes a stand is utilised as long as 25 years.

Seed sources: Standard and Fairway strains from N. Dakota.

Six sowings of three accessions of two strains were made from September to November. Germination was generally fair, with some loss of seedlings over the first winter. Plants from September sowing produced seed in reasonable quantity in the first season, and in general good seed could be harvested in the second year. The plants were tussocky in habit, the Standard and Fairway strains differing somewhat in leaf type. Foliage was abundant in spring, but by autumn it was sparse and poor, though some growth occurred late in the season. Many of the original plants persisted for six years or more, but there was a tendency towards gradual dying off.

Agropyron dasystachum

Thickspike wheatgrass is a sod-forming grass, producing good ground cover and satisfactory yields on light-textured soils. Growth begins early in spring. This has the same season of use as crested wheatgrass, and the two may be planted and grazed together.

Seed sources: the states of Washington and N. Dakota.

Eight sowings, seven of one accession, were made from September to November. Germination varied from fair to good, and was better from the later sowings. The species appeared to be resistant to frost, some growth having been observed as late as May. Seed was not produced until the second season after sowing and in some instances not until the third. In short summers seed did not always develop fully in this late-flowering species, and overall the seed crop was poor. Plants spread by means of extravaginal tillers, with an abundance of stems, but the total leaf production was poor and the habit rather spindly. Plants were still growing after seven years but tended to deteriorate in their central parts, while the tillers were more vigorous. One sowing was infected by a smut fungus which did not persist or spread to other trials.

Agropyron elongatum

Tall wheatgrass is a coarse, tall, vigorous, stemmy bunchgrass. It is more palatable than its appearance indicates and is one of the highest-yielding grasses yet tested in the United States. It is used in pure stands for soil and moisture conservation because of its special adaptations and its late summer growth. It is late in blooming, heading, and maturing. Seed germinates readily, but plants develop rather slowly, especially under semi-arid conditions. It is moderately self-fertile and seed production is excellent at medium altitudes when the growing season is long.

Seed source: N. Dakota.

Three sowings were made of one accession in September and November. Germination was only fair, and was poor when old seed was used, but there was little loss in the first winter. Flower heads appeared in the second season after sowing, at the end of January. Development of seed was sometimes arrested at an early stage at the onset of cold weather, and seed was collected in April only after a long dry summer. A robust tussock form with coarse wide leaves up to 24 in. long was developed by the second summer and was retained. Growth was slow in spring, and plants usually completely dried off by May. Occasional mowing did no harm, and there were no signs of deterioration after five or six years. Late seed set and rather poor germination were bad features.

Agropyron inerme

Beardless wheatgrass, indigenous to North America, produces a tougher sod, has a different season of use, and is more drought-resistant than crested wheatgrass.

Seed sources: the states of Washington and N. Dakota.

Seven sowings were made, five of one accession and one each of two others. Germination was generally poor, one sowing failed to germinate, and seedlings of two did not survive the first winter; of the others, one was discarded in the second season, and another, a very poor remnant, in the third. The remaining two still persisted after five years. Plants were tufted in habit, with numerous fine leaves. Seed did not appear until the second season and then in poor quantities. Only some of the plants were completely awnless and there was considerable range in the development of awns within individual sowings. This species in America requires three years to establish satisfactorily; here it barely makes enough growth in the short growing season to maintain itself over the winter.

Agropyron intermedium

Intermediate wheatgrass is perennial and sod-forming. Plants begin to grow in early spring and reach a height of 3–4 ft before growth ceases in midsummer. The return of moisture and cool temperatures in the late summer brings good growth recovery. The abundant foliage is relished by all classes of livestock, and growth is vigorous. A firm sod is somewhat less rapidly formed than in *Bromus inermis*. Excellent seedling vigour permits relatively low seeding rates.

Seed source: N. Dakota.

Eight sowings were made of three accessions, and germination was rather uniformly good. Growth began early in spring, about three weeks later than in *Bromus inermis*, and, once runners developed, the plants maintained themselves well over the winter with little tendency towards bareness in the centres. During the four to seven years of trials plants spread laterally a distance of 3 ft or more and produced an abundance of foliage with vigorous growth to a height of sometimes more than 4 ft. Leaves tended to be yellow except in the vicinity of lucerne plants. Seed production was rather erratic and well-filled seeds were the exception, the best being those from outer tillers. Apart from the fact that the species appeared to have good and bad flowering years, seed set depended on length and dryness of the summer and in a short damp season development ended at an early stage. Though there was some variation in the performance of different accessions, this was always one of the most attractive grasses in the nursery, but if these twitchy plants became established in a milder climate they could become unduly aggressive.

Agropyron sibericum

Siberian wheatgrass is closely related to Standard crested wheatgrass and is similar in appearance, except that the seed heads are narrower and the awns very short. Under semi-arid conditions, it has produced more foliage than Standard crested wheatgrass.

Seed source: Nebraska.

Three sowings were made of one accession: one failed to germinate, one did not survive the second winter, and plants from the third were still alive after four years. Seed was produced in the first year, ripening in March, but some of it was damaged by the weevil to which this species is susceptible. The tufted plants grew slowly and not very vigorously, and, with scant foliage, had little to recommend them.

Agropyron smithii

Western wheatgrass is a perennial, sod-forming grass distributed generally throughout the United States except in the humid south-east. Growth is vigorous, with seed heads at a height of 2–3 ft. It is hardy and drought-resistant, and has a capacity to spread rapidly by means of underground rhizomes. These underground parts make a profuse, dense growth, resulting in a tough fibrous sod that effectively binds the soil and offers protection from erosion. Seedlings are drought-resistant and compete fairly well with weeds and other grasses.

Seed source: N. Dakota.

Four sowings were made of two accessions. One germinated poorly and seedlings failed to survive the winter, but the others established fairly well and suffered little from frost. The strong rhizomes produced poor dry foliage. Growth began later than in *Agropyron intermedium* and plants were completely withered back by May. Seed production was patchy commencing in the second or third season. In single rows this species could not compete with invading *Poa pratensis* but it did so in wider strip sowings. Though persisting for as long as nine years, the plants deteriorated steadily, and the species was never attractive.

Agropyron spicatum

Bluebunch wheatgrass, a perennial, drought-resistant species, is found chiefly in the dry open areas of the western United States.

Seed source: N. Dakota.

Two sowings were made of one accession. One germinated well and the long even row still had a pleasing appearance after nine years. Seed was produced in the second year but never in any great quantity. Growth started fairly early in the season and plenty of foliage was produced. The second sowing gave a much poorer germination and plants were much less vigorous and uniform.

Agropyron trachycaulum

Slender wheatgrass is a perennial bunchgrass distributed throughout the United States except in the south-east and in the south central states. It commonly grows to a height of 3 ft and the dense leafy bunches may reach a foot or more in diameter, enlarging by tillering. Growth begins early in spring and the abundance of palatable forage is well liked by all classes of livestock. Although it occurs on most soil types, it prefers the lighter soils and sandy loams.

Seed sources: S. Dakota and Maryland.

Three sowings were made of two accessions. Germination was fair to good, with some slight frost losses. Vegetative growth started early in the season, but foliage produced was meagre and the plants somewhat strict in habit. Flowering sometimes occurred in the first year, but it was better in the second, the seed ripening in March. Deterioration began after the second year, though some plants survived six years or more.

Arrhenatherum elatius

Tall oatgrass has wide adaptation in semi-humid areas and under irrigation. The young plants are hardy and produce good ground cover, and older plants grow fast in the autumn and again in early spring. Root production is good. The foliage is palatable and recovers rapidly after grazing.

Seed source: Sweden.

One row of Swedish seed sown in October germinated and established well. In trials beyond the nursery, using seed from local sources, germination was almost uniformly good, and older plants have suppressed *Festuca novae-zelandiae* tussocks in more than one place. Described in the United States as palatable, tall oatgrass is rarely grazed at all at Molesworth. In sowings on the hills young plants were very closely cropped by rabbits, but only as long as there was a dearth of other green growth; many survived and have grown into large plants that produce quantities of light seed.

Bouteloua curtipendula

Sideoats grama is an erect perennial with coarse fibrous roots, strong creeping root-stocks, and rather leafy stems, 1–4 ft high. Despite the presence of root-stocks its growth is typically tufted. It grows vigorously, producing a considerable volume of leafage per plant and is a valuable forage species where sufficiently abundant.

Seed source: Kansas.

Two trials were made of two accessions: one failed to germinate; the other was poor, suffered heavy loss in the first winter, and died off completely in the second.

Bouteloua gracilis

Blue grama is a low-growing, long-lived perennial which grows throughout the Great Plains of the United States, where its capacity to resist drought permits it to occupy the drier sites. As a general rule seed should be used only near its point of origin. Growth begins fairly late in the season, and depends on how much moisture is available. The forage is relished by all classes of livestock.

Seed sources: Kansas and N. Dakota.

Six sowings of four accessions were made. Germination varied from poor to good, but plants were never strong and frost-heave resulted in gradual loss. Obviously the strains tested were not at all suited to Molesworth conditions.

Brachypodium pinnatum var. glabrum

Seed source: Canberra.

Two sowings were made of one accession of this false brome. Only a few poor plants of one survived the first winter, and a little seed was produced in the second year. In the milder conditions of Hutt Valley this plant was very aggressive and quite undesirable.

Bromus carinatus

California brome is a vigorous short-lived grass of the Rocky Mountain and Pacific Coast regions. Many botanists consider that this is a polymorphous species which includes also *B. marginatus* and *B. maritimus*. All are characterised by the capacity to produce large quantities of foliage that is relished by livestock while it is young; older leaves are harsh and less palatable, but this is offset somewhat by the fact that the seed heads are edible and nutritious.

Seed source: Wellington, New Zealand.

One sowing was made with good germination, and plants seeded well early in the season. Growth was at first good but plants died out almost entirely within five years. Volunteer plants appeared rather freely in various parts of the nursery.

Bromus inermis

Smooth brome is a long-lived, perennial sod-grass with strong creeping rhizomes, native to Europe, Siberia, and China. It was introduced into the United States some 75 years ago and has been widely grown, being adapted especially to regions of moderate rainfall and low to moderate summer temperatures. Two distinct types, differing in growth behaviour, are generally recognised. Growth begins early and continues through the summer if enough moisture is available. Its good volume of leafy forage is relished by all classes of livestock.

Seed sources: Achenbach, Elsberry, Lincoln Certified, Nebraska 36, and Nebraska 44 from Maryland; Fischer from Iowa; Mandan

404 from N. Dakota; Okla 2 Brome from Oklahoma, N. Dakota (part ex Manchuria), and Canberra (ex Germany, Pennsylvania, and Washington).

Seventeen sowings were made of 13 different accessions, including eight named strains. Germination was patchy. There was no loss from frost-heave, and an extensive system of runners soon developed. Seed was produced in the second year and ripened in March, and the seed set was usually good and uniform over the years. Seed is carried easily by wind and the species volunteered widely over the nursery. Leaves varied but were mostly broad, rather juicy, and numerous. This was one of the last plants to be frosted back, and growth began early, some weeks before *Agropyron intermedium*, and continued into May. Withered top foliage and straw protect the basal leaves. No disease or insect pest was noted, and trials which were under way for six years showed no sign of deteriorating; in fact, spreading from the rows continued during that time, with the formation of consolidated masses where little else could compete. Strains retained their distinctive characteristics, but there was some variation within strains. Some plants had a flat central portion which tended to be frosted, and, in some, growth began earlier.

Mixed seed harvested at Molesworth from two accession numbers was broadcast and harrowed in at the north end of the nursery in a block of about a quarter of an acre in October 1951. It established fairly well and has since been grazed. On the hills, in a few small areas near Molesworth homestead and in one above the lower Acheron River, this species has established from broadcast seed; these patches should be kept under observation to see if the grass is eaten by stock. A twitchy habit of growth, a tendency to become sod-bound, and light easily carried seeds are features that might make this a very unpopular plant if it migrated to better quality land.

Bromus marginatus

Mountain brome is a short-lived grass closely related to *B. carinatus* and similar to it in soil and climatic adaptation.

Seed source: Maryland.

Two sowings were made of one accession. Germination was very good, with little loss in the first winter. Flowers were produced in the first or second season in January and the seed was ripe by February. Seeding was heavy. Plants were strong, with yellow-green foliage, and grew well close together without forming tussocks. Growth began early and plants remained green late into the season with some autumn growth in April or even May. Rows tended to degenerate after the third seed year. This species compares with *Bromus carinatus* which also deteriorates and becomes weedy under Molesworth conditions. Three other species of this group, *B. maritimus, B. uruguayensis*, and *B. popovii*, the seed all from Canberra, behaved similarly over three years' observation, the last species having a slightly more luxuriant initial growth.

Buchloe dactyloides

Buffalo grass is a fine-leaved, semi-stoloniferous perennial. Generally it grows 4–6 in. high and spreads rapidly by surface runners to form a dense matted turf. Growth begins late in spring and continues through the summer. Because of its palatable foliage, excellent ground cover, aggressive spread under use, wide climatic adaptation, and relative ease of establishment, buffalo grass has been described in the United States as ideally suited for erosion control on range and pasture lands where soil does not contain too much sand.

Seed sources: hays from Kansas, N. Dakota, and Texas.

Seven sowings were made of five accessions. In all trials germination was good, but four failed to survive the first winter; one other was discarded in the second year, and the remaining two gradually disappeared over five years, after having produced only meagre stolons. Foliage was very susceptible to frost damage and the species was one of the first to brown off in autumn while root development, especially on stolons, was often insufficient to withstand frost-heave. Male and female flowers appeared on some of the older plants but no burrs were seen.

Dactylis woronowii

Seed source: Canberra.

Two sowings were made of one accession. One failed to germinate, perhaps because of poorly filled seed; in the other, germination was patchy and some plants were lost during the winter, but survivors made very good growth in the following season. Plants were robust and tussocky, with large lush leaves, growth was more vigorous than in the local strains of *D. glomerata*, and the foliage looked more succulent. Seed set in the second year, and the crop was good. Still another good feature was that volunteer plants appeared quite freely about the row within three years.

Ehrharta calycina

Perennial veld grass is native to the mountain districts of South Africa and has shown considerable promise as a winter-growing, drought-resistant grass in south-western Australia.

Seed source: Adelaide.

Four sowings were made of two accessions. Germination varied from poor to good and one trial developed as far as seeding; but within three years all succumbed to frost-heave.

Elymus canadensis

Canadian wild rye is a tall, rank, widely distributed, perennial bunchgrass. It begins growth about a week later in spring than crested wheatgrass, usually continues to grow throughout the summer if moisture conditions are favourable, and may resume growth in the autumn, after a summer drought, if enough moisture is available. The young seedlings are exceptionally vigorous and quickly form a good protective cover.

Seed source: N. Dakota.

Two sowings were made of two accessions. In both germination was good and subsequent growth vigorous, with harsh dry

foliage up to 1 ft high. Seed was produced in the first year, with abundant crops from the second year onwards, on culms to a height of 3 ft or more. Both accessions were under trial for six years and showed no sign of deterioration. The erect habit and coarse leaves made this look an unattractive plant, and its palatability was not tested.

Elymus glaucus

Blue wild rye is a perennial bunchgrass, growing throughout the western United States, particularly on old burns and cut-over areas in the north-west. It commonly grows in small tufts and rarely forms dense pure stands. It is most abundant on moist soils, but it stands considerable drought.

Seed source: Idaho.

Two sowings were made of two accessions. Germination was only fair, but the seedlings soon became firmly established. Growth began very early in the year and continued vigorously to produce plenty of coarse foliage, the leaves being broad in one strain and narrow in the other. Seed was set from the second season onwards. One trial was still growing strongly after nine years. Seedlings volunteering readily to some distance from the row produced few leaves and often flowered when very small. Little is known about the palatability of this species, and with its easily distributed seed it could become a weed.

Elymus virginicus

Virginia wild rye seed arrived as an impurity from Kansas, and one sowing was made. Germination was sporadic, but, once plants established, they set quantities of seed from the first year onwards. The foliage was poor and unthrifty, with abundant persistent old straw, while new growth was slow and sparse amongst the old dead stalks. Seedlings volunteered among adjacent rows and this species can only be regarded as weedy.

Eragrostis curvula

Weeping lovegrass is a vigorous perennial bunchgrass, native to South Africa. It is easily established from seed and the young seedlings quickly make an effective ground cover. The plants resist summer heat and drought, and survive temperatures as low as 11°F if the soil has adequate moisture at the time of the first killing frost.

Seed sources: New Mexico and N. Dakota.

Four sowings were made of two accessions, and, in all, germination was good; seedlings were particularly susceptible to frost-heave, and during the first winter plantlets up to 3 in. tall were thrown out, leaving the roots exposed. In the few surviving plants growth was fair and seed set well in the second year. Saxby (1957) records that in Central Otago this species established and grew well but was completely neglected when an area of it was opened up for grazing by sheep.

Eragrostis lehmanniana

Lehmann lovegrass is smaller than *E. curvula,* with less top growth and is drought-resistant and suitable for semi-arid regions. It is a most easily

established and rapidly-spreading layering perennial. After killing by prolonged drought it re-establishes from self-sown seed. It is palatable to horses, cattle, and sheep when green.

Seed sources: Arizona and N. Dakota.

Two sowings of one accession failed to germinate; one sowing of a different accession germinated quite well, but the seedlings were completely eliminated by frost-heave in the first winter.

Festuca elatior

Meadow fescue, a hardy perennial, flourishes in deep rich soils; in suitable places it will usually grow to 15–30 in. tall. It does not propagate itself by root-stocks or form a dense sod.

Seed sources: Alta, Justice, and Kentucky 31 from Maryland; unnamed strain from Oklahoma.

Five sowings of four different strains all germinated well. One trial sown in November was not sufficiently well established to survive the winter, but all the others made good growth over periods of up to five years, plants being tufted with abundant coarse green foliage. Growth began quite early in the season, some strains being earlier than others. In some, seed was developed in the first year and in all there was good seed set from the second year onwards. The closely related *F. arundinacea* (tall fescue) has long been well established in several areas a mile or two down-river from the nursery and there survives burning and grazing.

Festuca idahoensis

Idaho fescue is a densely tufted perennial grass 1–3 ft high, with many stems and fine narrow bluish-green leaves. Dried stubbly straw-coloured herbage from previous years usually contributes to the compactness of the bunch and is generally characteristic. It grows on all exposures and under a wide variety of soil conditions, but is most common on fairly dry, well drained, moderately deep, sandy or gravelly loams.

Seed source: Washington state.

Of three sowings of one accession one failed to germinate and the two others germinated poorly with some loss from frost-heave in the first winter. Abundant seed was set from the second year onwards. Plants were present after nine years but the compact clumps showed very little increase in size.

Festuca mairei

A first small sowing of seed from Canberra produced three plants which persisted, while a second sowing of the same accession resulted in a patchy row, where the plants were still quite good after three years and made growth late into the autumn. Leaves were yellow-green, and seed was produced in the second year in February.

Festuca ovina

Sheep fescue in two sowings of two accessions from Washington state germinated fairly well, but there was some loss by frost-heave in the first winter. Total growth was poor, though some of the tightly tufted plants persisted for nine years.

Festuca rubra

Seed sources: Canada and Oregon.

Two sowings of two strains of North American seed gave only fair germination, and performance generally was much as in *F. ovina*.

Poa ampla

Big bluegrass forms robust perennial tufted and vigorous plants about 2–4 ft tall. Though seldom found in dense stands, it is an important range species because of its heavy foliage production, palatability, and tendency to begin growth in early spring and continue into autumn. Because of its wide distribution, extensive root system, and adaptation to adverse sites and climates, it is useful for regrassing abandoned farm land and depleted rangeland.

Seed source: Oregon.

Five sowings were made of two accessions. Three failed to germinate and plants from the others increased little in size over a period of six years. In one trial seed ripened in March of the first year.

Poa compressa

This bluegrass is a hardy perennial which is very persistent when established, will do better than *Poa pratensis* on the poorer and drier sites, and does not form so dense a sod. Tests indicate that this species has potential value for reseeding certain depleted or badly eroded areas where precipitation is adequate and temperatures are cool.

Seed source: Ottawa.

Three sowings were made of two accessions. Both germination and survival over the winter were good. Seed was produced in the first year, ripening in January. There was no trouble with frost-heave. Growth started very early in the season, but was rather slow and poor, and the small clumps of plants produced over two years of observation were rather unimpressive.

Secale cereale

Seed source: seeds of unrecorded, reputedly hybrid, origin.

A first sowing of seed from Waiwhetu germinated well and produced plants which survived for up to two years, producing plenty of grain. A row from this Molesworth seed produced good seed, especially in its second year, and many of the plants persisted, with some seed, to the fourth year. Some seedlings volunteered and seeded far from the parent row.

Stipa viridula

Green needlegrass is a rather coarse leafy perennial, abundant on the upland prairies of the northern Great Plains of the U.S. It seems to be well adapted to many soil types but makes its best growth on the sandier soils. It grows to 3 ft tall and has deep penetrating roots. Growth starts early in spring and continues into autumn when enough moisture is available. It makes excellent recovery after grazing and clipping, and provides good forage for all classes of livestock. Young seedlings are vigorous and fairly resistant to drought and insects.

Seed source: N. Dakota.

Three sowings of one accession were made in October and November. Germination was mostly good, but some was only fair. Foliage was little damaged by frost, but young plants were shallow-rooted and very susceptible to frost-heave in the first winter. Plants were erect, strict in habit, with numerous leaves, both narrow and broad. Seed matured in February and growth ended early in autumn. Plants from one sowing were still growing after six years but the performance was mediocre.

Other Species

Species which were sown but failed to establish successfully were (with seed sources): *Agropyron desertorum* and *A. glaucum* (Canberra); *A. scabrifolium* (Uruguay); *Eragrostis plana* (Pretoria); *E. trichodes, Panicum virgatum,* and *Poa secunda* (N. Dakota); *Sporobolus airoides* (Oklahoma); *S. cryptandrus* (Kansas).

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A STUDY OF THE GROWTH AND DEVELOPMENT OF JERSEY CATTLE

I. A NEW CARCASS DISSECTION TECHNIQUE

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Summary

A new technique for use in studies involving carcass dissection is described and illustrated. The advantages of using this technique, particularly for cattle carcasses, are discussed. This preliminary paper is the first of a series detailing the results of a growth and development study of cattle.

Introduction

In studying the growth and development of farm animals, many workers have made use of a dissection technique entailing a separation of the various main tissues of the body (Hammond 1932; Palsson 1939; McMeekan 1940; Walker and McMeekan 1944). Most of this work has been concerned with the carcass composition of meat animals. When the smaller species, such as sheep and pigs, have been involved, whole or half carcasses have frequently been completely dissected, but when cattle have been under study a "sample joint" has more often been used on account of the expense and labour involved (Hankins and Howe 1946).

To provide information on regional growth, and also to facilitate handling, sheep and pig carcasses have been broken down initially in the laboratory into "joints" which have been treated as dissection units. These "joints" have seldom been those recognised by the meat trade, in which the accuracy of cutting, even by a skilled tradesman, is not consistent enough for scientific purposes. Laboratory cutting has been based more on fixed carcass points by which a "joint" could be accurately defined. Nevertheless, for practical purposes, these "joints" have borne a loose relation to the butchers' joints. This has been useful when meat type animals have been under study.

The procedure has been quite satisfactory in the hands of experienced operators, and valuable data on differential growth have been obtained by this approach. The larger the carcass, however, the more difficult it is to break down accurately, and the more important it becomes to break it down in some way to avoid

Text continued on page 107

Fig. 2.—Stripped carcass ready for dissection. 1—Trapezius. 2—Brachiocephalicus. 3—Omo-transversarius. 4—Sterno-cephalicus. 7—Supraspinatus. 8—Infraspinatus. 9—Tensor fasciae antibrachii. 10a, b, c—Triceps. 11—Deltoid. 12—Brachialis. 13—Flexor group—fore. 14—Extensor group—fore. 15—Latissimus dorsi. 16—Deep Pectoral. 17—Superficial Pectoral. 18—Serratus ventralis-thoracic part. 19a, b, c—Serratus dorsalis posterior. 21—Obliquus abdominis externus. 22—Obliquus abdominis internus. 23—Rectus abdominis and aponeurosis. 25—Longissimus dorsi. 27—Multifidus dorsi. 29—Biceps femoris. 30—Tensor fasciae latae. 31—Gluteus medius. 32—Quadriceps femoris. 33—Semitendinosus. 35—Gastrocnemius. 36—Flexor group—hind. 37—Lateral extensor. 38—Peroneus longus. 39—Peroneus tertius and Long extensor group. 41—Soleus.

Fig. 3.—Carcass with shoulder and some first-layer muscles removed. 4—Sterno-cephalicus. 6—Omo-hyoideus. 16—Deep Pectoral. 17—Superficial Pectoral. 18—Serratus ventralis-thoracic part. 18a—Serratus ventralis-cervical part. 19a, b, c—Serratus dorsalis posterior. 20—Rhomboideus. 21—Obliquus abdominis externus. 22—Obliquus abdominis internus 23—Rectus abdominis. 24—Transversus abdominis. 25—Longissimus dorsi. 26—Longissimus costarum. 27—Multifidus dorsi. 28—Intercostals. 31—Gluteus medius. 32—Quadriceps femoris. 33—Semitendinosus. 34—Semimembranosus. 35—Gastrocnemius. 36—Flexor group—hind. 37—Lateral extensor. 38—Peroneus longus. 39—Peroneus tertius and Long extensor group. 40—Tibialis anterior. 41—Soleus. 42—Splenius. 43—Longissimus capitis et atlantis. 44—Scalenus ventralis. 45—Scalenus dorsalis. 46—"Subclavius". 51a.—Iliacus. 54—Gluteus profundus. 55—Quadratus femoris. 56—Adductor. 65—Gemellus. 68—Intertransversales. 69—Rectus capitis ventralis major.

Fig. 4.—Carcass, showing deeper muscles. 24—Transversus abdominis. 25—Longissimus dorsi. 25a—Longissimus dorsi-cervical part. 26—Longissimus costarum. 27—Multifidus dorsi. 28—Intercostals. 34—Semimembranosus. 35—Gastrocnemius. 36—Flexor group—hind. 37—Lateral extensor. 38—Peroneus longus. 39—Peroneus tertius and Long extensor group. 40—Tibialis anterior. 41—Soleus. 42—Splenius. 43—Longissimus capitis et atlantis. 44—Scalenus ventralis. 47—Complexus. 50—Serratus dorsalis anterior. 51a—Iliacus. 54—Gluteus profundus. 55—Quadratus femoris. 56—Adductor. 60—Rectus thoracis. 61—Gluteus accessorius. 62—Sartorius. 64—Pectineus. 65—Gemellus. 68—Intertransversales. 69—Rectus capitis ventralis major. 76—Ligamentum nuchae.

Fig. 5.—Carcass, showing medial thigh, sublumbar, and deep neck muscles. 27—Multifidus dorsi. 30—Tensor fasciae latae. 32—Quadriceps femoris. 33—Semitendinosus. 34—Semimembranosus. 35—Gastrocnemius. 36—Flexor group—hind. 39—Peroneus tertius and Long extensor group. 43—Longissimus capitis et atlantis. 47—Complexus. 51—Psoas major } Ilio-psoas major 52—Psoas minor.
51a—Iliacus }
53—Quadratus lumborum. 62—Sartorius. 63—Gracilis. 64—Pectineus. 66—Obturator externus. 68—Intertransversales. 73—Longus colli. 74—Transversus thoracis. 76—Ligamentum nuchae. 77—Diaphragm.

Fig. 6.—Carcass, showing deep muscles of thigh and neck. 27—Multifidus dorsi 28—Intercostals. 30—Tensor fasciae latae. 32—Quadriceps femoris. 33—Semitendinosus. 34—Semimembranosus. 35—Gastrocnemius. 36—Flexor group—hind. 39—Peroneus tertius and Long extensor group. 51—Psoas major } Ilio-psoas major
51a—Iliacus }
52—Psoas minor. 53—Quadratus lumborum. 56—Adductor. 62—Sartorius. 64—Pectineus. 66—Obturator externus. 68—Intertransversales. 70—Obliquus capitis posterior. 71—Rectus capitis dorsalis major. 72—Multifidus cervicis. 73—Longus colli. 74—Transversus thoracis. 76—Ligamentum nuchae. 77—Diaphragm (cut).

Fig. 1.—The carcass of a 2-year-old Jersey heifer. P—Panniculus carnosus.

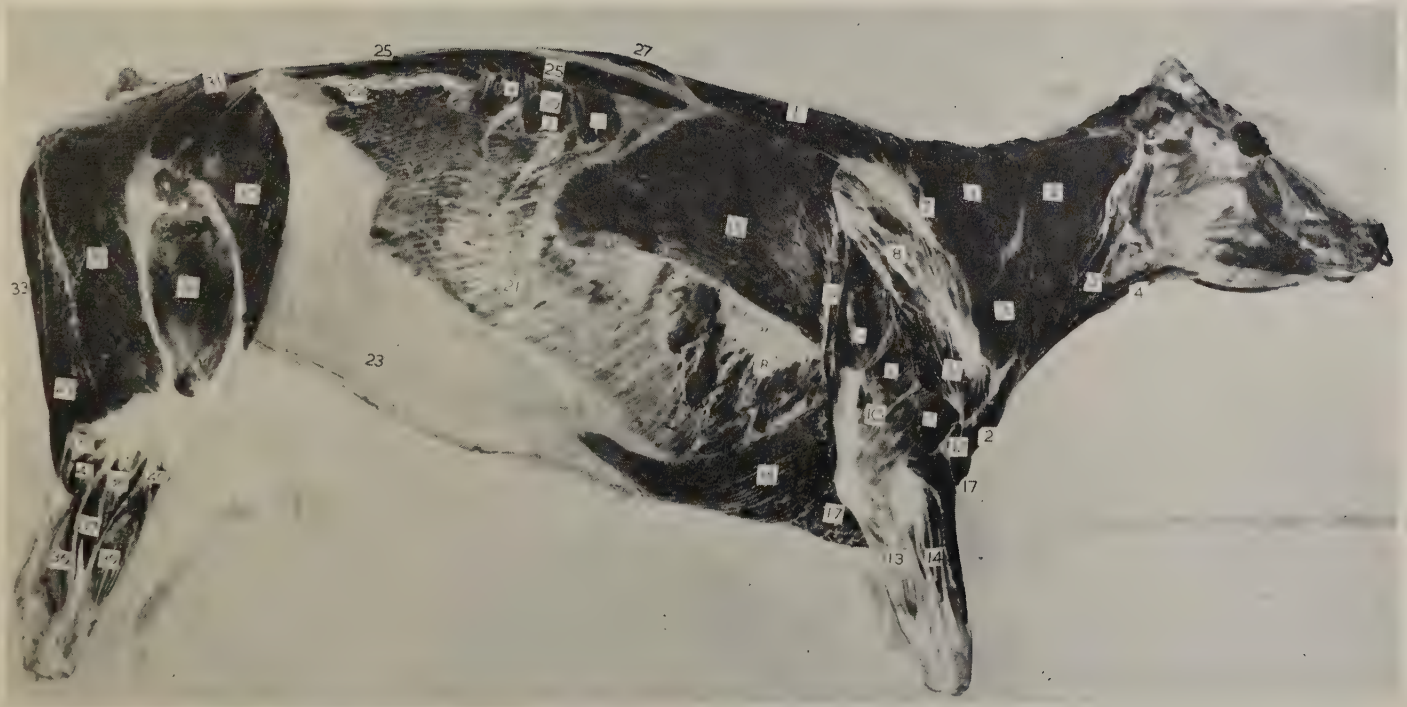

Fig. 2.—Stripped carcass ready for dissection. (Details are given on page 100.)

Fig. 3.—Carcass with shoulder and some first-layer muscles removed. (Details are given on page 100.)

Fig. 4.—Carcass, showing deeper muscles. (Details are given on page 100.)

Fig. 5.—Carcass, showing medial thigh, sublumbar, and deep neck muscles. (Details are given on page 100.)

Fig. 6.—Carcass, showing deep muscles of thigh and neck. (Details are given on page 100.)

moisture loss and decomposition as work proceeds. With cattle beasts, size, weight, and conformation all militate against accurate jointing by any method. In non-meat animals "joints" as such obviously have little application. Hence, at the Ruakura Animal Research Station, when a study of growth and development in Jersey cattle was contemplated on the basis of complete dissection of one side of the carcass, a new approach was sought.

METHOD

It was decided that handling difficulties could be overcome and that more specific and accurate information could be obtained by dissecting the carcasses on an individual muscle and bone basis. This technique was originally adopted for a study of growth and development in Jersey cattle which involved, in all, some 60 pairs of identical twins varying in age from two days to five years. It has now become the standard technique used at Ruakura in cattle studies and, in certain circumstances, in studies involving other species.

The method used in the ultimate separation of the tissues followed the general pattern used by the Cambridge school of workers (Palsson 1939; McMeekan 1940). Every effort was made to avoid moisture losses. The work was carried out as quickly as possible, and the carcass and its parts were subjected to the minimum of handling. However, in the interests of speed, handling was allowed during the cleaning of organs and also to some extent while the carcass was being dissected, but all work thereafter was done with forceps, scalpels, and scissors. Except for the areas actually being worked on, the carcass and its parts were kept covered with towels which had been soaked in cold water and then wrung damp dry. Muscles and bones awaiting cleaning were laid on damp towels in a refrigerator running at 34°F, and covered with other damp towels. Immediately any part was cleaned it was weighed, then set aside in the refrigerator for check weighing at the end of the day. The fat, tendon, etc., removed in cleaning, was collected at frequent intervals and weighed.

Preliminary Procedure

Each animal was weighed, then fasted overnight and re-weighed before slaughter in the morning. Killing was carried out with a captive bolt humane killer and the throat was subsequently slit in the normal manner. Blood was collected by drainage and by swabbing with cotton wool, the swabs being weighed with the blood. The body was then flayed, head and feet being cut off in the process. The right feet and the head were put aside for laboratory dissection. Any adhering subcutaneous fat and muscle fragments were removed from the right side of the hide and weighed, the muscle fragments being first identified so that their weight could eventually be added to that of the muscle to which they belonged. Data on organ weights were collected at the abattoir. Organs were removed by the normal dressing procedure, and were trimmed of fat etc. before they were weighed. All internal fat was collected

Fig. 7.—Shoulder. Lateral view.

7—Supraspinatus. 10a, b—Triceps. 12—Brachialis. 13—Flexor group—fore. 14—Extensor group—fore. 48—Biceps brachii. 49—Teres minor. 57—Teres major.

Fig. 8.—Shoulder. Medial view.

7—Supraspinatus. 9—Tensor fasciae antibrachii. 10, b—Triceps. 12—Brachialis. 13—Flexor group—fore. 14—Extensor group—fore. 48—Biceps brachii. 57—Teres major. 58—Coraco-brachialis. 59, a, a—Subscapularis.

and weighed. The various sections of the alimentary tract were cleaned of fat, emptied, thoroughly washed under running water, and then allowed to drain from hooks before weighing. The carcass was weighed and conveyed whole to the laboratory for dissection

Laboratory Procedure

Before dissection was begun, the udder or cod fat was removed and weighed and the tail cut off, using the standard butchers' cut which follows up the posterior border of the sacro-sciatic ligament and divides the coccygeal vertebrae from the sacrum. Subcutaneous fat from around the tail head was removed, that from the right side weighed, and the tail weighed and discarded without further dissection.

Figs. 1–6 show the carcass of a two-year-old Jersey heifer at various stages during dissection. The head has been left on to show the points of attachment of the neck muscles. The fore limb is shown as a separate unit in Figs. 7 and 8, and the individual muscles are pictured in groups in Figs. 9–21. The nomenclature used is that of Sisson and Grossman (1940).

The muscles are removed individually and as expeditiously as possible. Most present little difficulty, the deep muscles of the neck being the most complicated. In two cases, arbitrary divisions are made. It will be found almost impossible to separate the Longus colli from the Intertransversales of the neck with any accuracy, so division is made by drawing a knife between the transverse processes of the cervical vertebrae from the posterior point of one to

Fig. 9.—Ventral muscles of the neck. 2—Brachiocephalicus. 6—Omohyoideus. 46—"Subclavius". 68—Intertransversales colli. 45—Scalenus dorsalis. 44—Scalenus ventralis. 4—Sterno-cephalicus. 69—Rectus capitis ventralis major. 73—Longus colli.

the anterior point of the next. The only difficult separation in the hindquarter is dealt with in a similar fashion. The fibres of the Obturator externus and the Obturator internus are so intermingled as to be almost inseparable. Division between these two muscles is, therefore, made by drawing a knife externally across the obturator foramen.

In some cases where the work involved does not seem warranted for the slight additional information which would be gained, closely related muscle groups are not separated. This applies in the case of the costal muscles where the Retractor costae, the Levatores costae, and the Intercostals are all weighed together. The Flexors and Extensors of the fore limb are not subdivided, nor are the Flexors of the hind limb. The Intertransversales of the back are taken out and weighed with the Multifidus dorsi to avoid a tedious separation.

In the neck region some muscles are necessarily mutilated when the throat is cut and the head removed by normal slaughtering methods. When the bulk of the muscles concerned remains on the carcass, weights are recorded, but in the circumstances over-meticulous separation cannot be justified. Hence, the Rectus capitis ventralis minor is weighed with the Rectus capitis ventralis major, and the Sterno-thyro-hyoideus, which is mutilated when the throat is slit, is included in the Sterno-cephalicus. When only small

Fig. 10.—Lateral muscles of the neck. 42 Splenius. 71—Rectus capitis dorsalis major. 47—Complexus. 70—Obliquus capitis posterior. 3—Omotransversarius. 43—Longissimus capitis et atlantis. 72—Multifidus cervicis.

Fig. 11.—Muscles of the thorax. 77 (upper)—Diaphragm. 77 (lower)—Diaphragm. 28—Intercostal. 74—Transversus thoracis (sternal border uppermost). 60—Rectus thoracis.

fragments of muscles remain on the carcass they are weighed collectively as "muscle fragments", e.g., the Retractor ani, Rectus capitis dorsalis minor, and Obliquus capitis anterior. Also included under this heading are the small fragments unavoidably left on the bones in the course of dissection.

The work of breaking down the body can proceed simultaneously on the fore-end and the hind-end. It is difficult to give any specific order for the removal of the muscles, particularly in the latter stages. If the body has been "soft-sided,"* the kidney and channel fat, the medial muscles of the thigh, sublumbar muscles, diaphragm, Transversus abdominis, and Transversus thoracis can be much more conveniently taken out at an earlier stage than if the body is whole. The relative speed and skill of the members of the dissection team will also affect the order. However, with experience a workable routine is soon established. The following illustrations and description give an approximate order of working.

The tail and udder have been cut off from the carcass shown in Fig. 1, but before dissection can be started the Panniculus carnosus muscle (P) and the subcutaneous fat must be stripped off. The fat under the Panniculus carnosus is classed as subcutaneous. Stripping the body takes some time, and as this work proceeds the fat is amassed in damp towels and weighed at frequent intervals.

* See Discussion.

Fig. 2 shows the stripped carcass ready for dissection.

On the fore-end the Trapezius (1) is easily removed. The Brachiocephalicus (2) is then lifted back from its origins and freed from the Omo-transversarius (3) and a small muscle described by Sisson and Grossman (1940) as the "homologue of the Subclavius in man" (46, Fig. 3). The Brachiocephalicus is left attached to the shoulder at this stage. The Omo-transversarius (3) and the Latissimus dorsi (15) are taken off next, although it may not be possible completely to free the latter until the shoulder has been

Fig. 12.—Muscles of the back and loins. 27—Multifidus dorsi. 19—Serratus dorsalis posterior. 50—Serratus dorsalis anterior. 25—Longissimus dorsi. 25 (a)—Longissimus dorsi—cervical part. 26—Longissimus costarum.

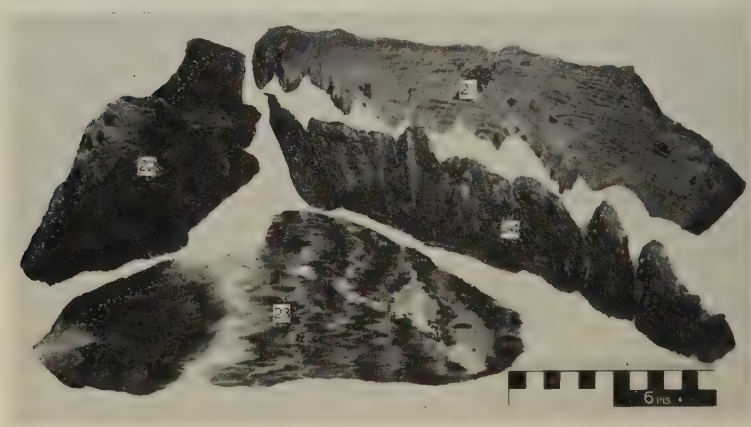

Fig. 13.—Abdominal muscles. 21—Obliquus abdominis externus. 22—Obliquus abdominis internus. 23—Rectus abdominis. 24—Transversus abdominis.

lifted. The Superficial and Deep Pectoral Muscles (17 and 16) and the Rhomboideus (20, Fig. 3) are partially freed from their shoulder insertions, after which the shoulder is lifted and completely freed from them and from the cervical part of the Serratus ventralis muscle (18a, Fig. 3) for dissection as a separate unit (Figs. 7 and 8).

The first muscle to be removed from the shoulder is the Deltoid (11, Fig. 2), the two parts of which are most easily detached by lifting the muscle from its insertion to the humerus. The Tensor fasciae antibrachii (9, Figs. 2 and 8) is then stripped off from the posterior edge of the long head of the Triceps (10). The bulk of the Infraspinatus (8, Fig. 2) lies in the infraspinous fossa of the scapula, but the posterior distal part overlies the Teres minor (49, Fig. 7), and the separation needs some care. The Supraspinatus (7, Figs. 7 and 8) is clearly defined by its relations to the spine of the scapula and to the Subscapularis (59, a,a, Fig. 8). Teres major (57, Fig. 8) passes between the Coraco-brachialis (58, Fig. 8) and the medial head of the Triceps brachii (10, b, Fig. 8) for its insertion to the humerus. The Anconeus, which covers the olecranon fossa, is difficult to separate from the lateral head of the Triceps and is, therefore, included with it. The Biceps brachii (48, Figs. 7 and 8) is a neat fusiform muscle which is easily detached. There remain only the Brachialis (12, Figs. 7 and 8), twisting round the musculo-

Fig. 14 (a).—Muscles of the shoulder girdle. 20—Rhomboideus. 1—Trapezius. 15—Latissimus dorsi.

Fig. 14 (b).—Muscles of the shoulder girdle. 18—Serratus ventralis (thoracic part). 18a.—Serratus ventralis (cervical part). 17—Superficial Pectoral. 16—Deep Pectoral.

Fig. 15.—Muscles of the shoulder. 59—Subscapularis. 8—Infraspinatus. 7—Supraspinatus. 57—Teres major. 58—Coraco-brachialis. 49—Teres minor. 11—Deltoid.

spiral grove of the humerus, and the groups of Flexors (13) and Extensors (14), to be removed.

From the hindquarter the Tensor fasciae latae (30, Fig. 2) and the Biceps femoris (29) are easily removed, although the head of the Semitendinosus (33) may have to be lifted before the latter can be detached from the tuber ischii. Fig. 3 shows the carcass at this stage. The Gluteus medius (31) and the Semitendinosus (33) are then dissected out, and the Quadriceps femoris (32) can be partially freed. It is more expeditious to separate the patella from the latter during the "cleaning" process. From the lower leg the Soleus (41), Lateral extensor (37), Peroneus longus (38), Peroneus tertius and extensor group (39), and the Tibialis anterior (40) can be removed.

On the fore-end the Superficial Pectoral (17) and the Deep Pectoral (16) are easily detached. The thoracic part of the Rhomboideus (20) is free, but the cervical part is united with its counterpart on the left side of the body and must be carefully divided. The two parts of the large Serratus ventralis muscle (18) are then lifted, the cervical part (18a) probably requiring the concurrent removal of the Scalenus dorsalis (45).

The Sterno-cephalicus (4), Omo-hyoideus (6), and "Subclavius" (46) present no difficulties. From the back the Serratus dorsalis posterior (19a, b, c) is taken off. In the abdominal region the Obliquus abdominis externus (21) the Rectus abdominis (23) and the Obliquus abdominis internus (22) are all accessible for removal. As much of the aponeurosis as can be lifted without damaging the

Fig. 16.—Muscles of the arm. 9—Tensor fasciae antibrachii. 10—Triceps brachii. 12—Brachialis. 48—Biceps brachii.

Fig. 17.—Muscles of the forearm. 13—Flexor group. 14—Extensor group.

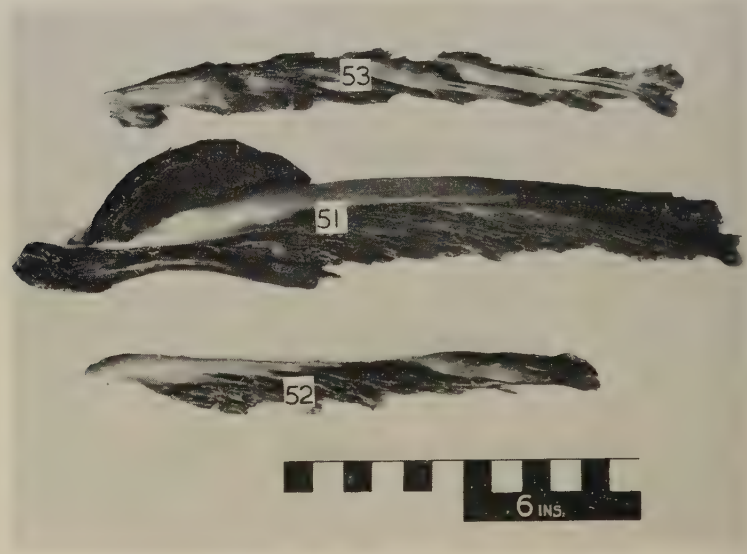

Fig. 18.—Sublumbar muscles. 53—Quadratus lumborum. 51—Ilio-psoas major. 52—Psoas minor.

muscles is cleaned of fat and weighed separately, to be subsequently included with tendon and waste. At any convenient stage, usually after the removal of the first two abdominal muscles named above (if the whole carcass is on the table), the kidney and channel fat is taken out.

This leaves the carcass as pictured in Fig. 4. Four small muscles closely related to the pelvis and femur are taken off. These are the Gluteus accessorius (61), Gluteus profundus (54), Gemellus (65), and Quadratus femoris (55). From the lower leg the Gastrocnemius (35) and the Flexor group (36) can be partially detached. On the fore-end the Splenius (42) is next removed from the neck and the Serratus dorsalis anterior (50) and the Rectus thoracis (60) from the thorax. From the ventral region of the neck the Rectus capitis ventralis major (69) is taken out from between the Intertransversales (68) and the Longus Colli, and the Scalenus ventralis (44) is freed from its attachment to the first rib and lifted off. The Longissimus costarum (26) and Longissimus dorsi (25) can be taken off. The cervical part of the latter is independent and requires patient dissection to separate it from the dorsal row of the Intertransversales (68) of the neck. This is most easily achieved by lifting the muscle from its thoracic attachments. In the lumbar region the intertransverse ligament between the lateral processes should be left intact to ensure that the sub-lumbar muscles are not damaged, unless a half carcass is being handled, in which case it is preferable to take out these muscles earlier.

Fig. 19.—Lateral muscles of hip and thigh. 34—Semimembranosus. 33—Semitendinosus. 29—Biceps femoris. 31—Gluteus medius. 30—Tensor fasciae latae. 61—Gluteus accessorius. 54—Gluteus profundus. 65—Gemellus.

The removal of the Longissimus capitis et atlantis (43) and the Complexus (47, Fig. 5) exposes the deepest muscles of the neck (Fig. 6). Rectus capitis dorsalis major (71) and Obliquus capitis posterior (70) present no difficulties. The division between the Multifidus cervicis (72) and the Multifidus dorsi (27) is not always very clear. The Intertransversales (68) of the neck have to be excavated from between the vertebral processes. The arbitrary method of separating them from the cervical part of the Longus colli (73) has already been described. The only remaining muscle on the back is the Multifidus dorsi (27), with which are taken the Intertransversales of the back.

Before the Intercostals (28) can be cut out, the rib cage must be lifted and the diaphragm (77), the Transversus abdominis (24, Fig. 4), and the Transversus thoracis (74, Fig. 6) removed. Provided that care is exercised in disjointing the ribs, the Longus colli (73) can be left at this stage and more easily removed from the vertebrae later. In Figs. 5 and 6 the ribs and femur of the right side have been sawn off to show these muscles, the sub-lumbar muscles, and the medial muscles of the thigh on the left side.

If the sub-lumbar muscles, the Ilio-psoas major (51), Psoas minor (52), and Quadratus lumborum (53) have not been already removed, they can now be taken out. To expedite the removal of the Intercostals a knife can be drawn at right angles close to the

Fig. 20.—Medial and anterior muscles of thigh. 63—Gracilis. 64—Pectineus. 62—Sartorius. 66—Obturator externus. 67—Obturator internus. 55—Quadratus femoris. 56—Adductor. 32—Quadriceps femoris.

bone down the edges of each rib. When the ribs are cleaned the weight of the muscle fragments attached to them is added to the Intercostals' weight.

With the bulk of the muscles no longer attached, the hind leg can be raised to facilitate the removal of the medial muscles. The Gracilis (63, Fig. 5), Sartorius (62), and Pectineus (64) are easily detached. It will usually be found more convenient to take off the Semimembranosus (34, Fig. 6) and the Adductor (56) together and separate them afterwards. The removal of the Quadriceps femoris (32), the Gastrocnemius (35), and the Flexor group (36) can be completed. This leaves only the Obturator externus (66) and the Obturator internus attached to the pelvis. The method of separation of these two has been described on page 110. Parts of the Retractor ani and Coccygeus muscles still attached to the pelvis adjacent to the Obturator internus are weighed with "muscle fragments".

When all muscles have been removed the bones are detached and set aside for cleaning. The vertebral column is taken out in sections after the muscles of the left side have been cut away and the left ribs sawn through about 2 in. below the heads—if the whole carcass is on the table.

In cleaning the bones of the limbs the first step is to remove fat, tendon, and muscle fragments; the bones are then scraped down, removing the periosteum. These scrapings are weighed with

Fig. 21—Muscles of the leg and foot. 35—Gastrocnemius. 41—Soleus. 36—Flexor group (hind). 37—Lateral extensor. 38—Peroneus longus. 39—Peroneus tertius and Long extensor group. 40—Tibialis anterior.

the tendon and waste. With the vertebrae, the cleanings from the left side are discarded. The fat and any tendon from the right side is weighed with other intermuscular fat and tendon. The bone scrapings are weighed separately from each section as a check on cleanness of dissection, but these weights are eventually added to the "muscle fragment" weight in accordance with the technique used for meat animals, in which case it is argued that this material is largely edible and little tendon is involved. The spinal cord and the fat surrounding it is taken out finally. As far as possible, the same section of the vertebrae from each beast was cleaned by the same assistant with a view to standardising the treatment, as these are the most difficult bones to deal with. The ribs and the sternum are treated similarly to the vertebrae. The muscles are comparatively simple to clean. Intermuscular fat and fasciae are removed from the surfaces, and tendons are cut off squarely at the limit of the muscle to which they belong. All fasciae are weighed with the intermuscular fat since they are in large part inseparable from it.

In dealing with the head, no attempt is made to dissect out the muscles. The tongue is removed, given a standard trim, and weighed. The head is skinned and the pelt weight added to the main pelt weight. The eyes, brain, and pituitary are taken out, cleaned, and weighed. The jaw bone and hyoid bones are detached and are scraped as clean as possible without differentiating between the various tissues. The skull is treated similarly. All soft tissues are weighed together as "head waste."

Organisation

Dissection on this scale is naturally time-consuming. On the average, with efficient organisation, a two-year-old heifer can be completely dissected in about 14–16 hours by a team composed of a leader and two trained technicians, assisted by 8–10 casual workers with varying degrees of experience, working approximately 12 hours of the above period cleaning the muscles and bones. This covers the time spent on organs at the slaughter-house. Younger cattle require less time, six-month-old beasts taking only a day.

The routine followed when dissection was to be done immediately after slaughter was for the beast to be killed at 7 a.m. in the presence of the leader and two technicians, who collected the blood. The body was carefully flayed by the butcher, then the hide was attended to. While the carcass was being dressed, the right fore and hind feet were skinned and the bones were separated. The component parts of each foot were wrapped in a damp towel and sent to the laboratory where they awaited the few other members of the team who started work at 8 a.m. As the body was eviscerated the organs were cleaned and, in the case of the digestive tract, washed out, then weighed. Two casual workers started at 8 a.m. to assist with this work. They remained with one technician until the job was completed, usually at about 9.30 a.m. The leader and the other technician followed the carcass to the laboratory at 9 a.m. and immediately began the task of stripping off the subcutaneous fat. Two of the more experienced casual workers assisted with

this work, which usually took approximately an hour and a half on a two-year-old Jersey heifer.

During this time the rest of the team, some of whom did not start work till 9 a.m., were kept busy dealing with the feet and the Panniculus carnosus. If there was not sufficient material ready to keep the cleaning team occupied, some of the superficial muscles were removed as soon as the area surrounding them had been cleared of subcutaneous fat. On occasions the removal of the subcutaneous fat from these muscles, e.g., Trapezius and Latissimus dorsi, was left to the cleaning team after the fat limits had been marked by scalpel cuts where necessary. However, to avoid any confusion, usually the subcutaneous fat was stripped from the whole side before any muscles were lifted. As each muscle was taken off, the appropriate typed label, from a sheet which had been prepared in advance, was placed on it and the muscle was either handed out for immediate cleaning or placed in the refrigerator until required. One technician was put in charge of the weighing, the handing out of material from the refrigerator, and the supervision of the cleaning work.

Discussion

In view of the amount of work entailed, only the right side of each beast was dissected, except for the head and the vertebral column which were removed complete. Hence, although the carcass could not be "backed down" in the normal way, the greater part of the left side could have been carefully cut away at the abattoir had the value of the meat warranted it. The carcasses were transferred to the laboratory before cooling down, and since meat cut from a hot carcass is unattractive in appearance and difficult to handle, and the value in these cases was low, no attempt was made to break down the carcasses to salvage edible meat in the half not being dissected. Later, when more valuable meat animals were under study by the same technique, they were "soft-sided," i.e., the soft tissues of the left side were carefully cut away from the vertebral column, then the left side of the pelvis and the left ribs were sawn through so that the whole of the vertebral column remained with the right side. This procedure on the whole is preferable, since the "half" carcass is more easily handled on the dissection table and can be turned over at any stage to remove the sub-lumbar muscles, the medial muscles of the thigh, and those muscles which are attached inside the rib cage.

It will be noted that for this work the carcasses were dissected before cooling. Dissection can be carried out much more easily and quickly when the carcass is in this state or merely cooled. In later projects frozen carcasses were handled, and these were much more difficult to break down and took much longer. The optimum time required for thawing out is difficult to judge. Some of the muscles tend to become mushy and difficult to dissect out on account of their fragility. In heavy carcasses particularly, the more superficial muscles may reach this stage while the deeper-seated ones are still brittle with ice crystals and therefore difficult to seam out accur-

ately. Although "drip" can be weighed, and thus accounted for, the unmeasurable losses during dissection are greater with such carcasses. The smallest losses should occur when the carcasses have been cooled or chilled.

Although the use of this technique places rather more of a burden on the leaders of a dissection team than does the jointing method, the work of the assistants is greatly simplified. Instead of being presented with heterogeneous lumps of muscle, fat and tendon, in the separation of which they must make numerous small decisions, they are only called upon to remove superficial fat from each muscle entity, whilst tendons, where present, are so obvious in their attachments as to give no cause for confusion. Consequently there is less scope for error, and less instruction and supervision is necessary, yet results are more accurate. The muscles soon come to be recognised and dealt with more quickly and in a standard fashion.

This work is naturally somewhat tedious, but it is obvious that a much greater interest is evinced when the carcass is broken down in this manner because the units have some individuality. The more specific type of information which results will be obvious from succeeding papers, which will deal primarily with the growth and development of Jersey cattle. These will also discuss the possibilities of using sample muscles and bones as indices of the whole. Such aspects are a matter of considerable importance in view of the expense and time involved in studies of this nature.

Acknowledgments

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VERTICILLIUM WILT OF TOBACCO

III. THE EFFECT OF CULTURE FILTRATES OF *Verticillium dahliae* Kleb. ON TOBACCO

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Summary

Culture filtrates of *Verticillium dahliae* grown on synthetic liquid media caused wilting in tobacco seedlings of varieties susceptible to this disease but not in those showing some field resistance.

The best toxin production was obtained on a modified Czapek's medium used in shake flask culture.

Dilution and heat sterilisation reduced the wilt-producing capacity of culture filtrates.

Isolates of *V. dahliae* from *N. tabacum* produced more toxin than those from *N. sanderae*, or *Rubus ideaus*, while toxin from *V. albo atrum* failed to produce wilting in tobacco.

Introduction

The growing of tobacco plants, in fields or glasshouses, in soil heavily inoculated with *Verticillium dahliae* (Thomson and McLeod 1959) has not shown any reliable differentiation in resistance between tobacco varieties or *Nicotiana* species (McLeod and Thomson 1959). One of the main disadvantages of this method was that wilting symptoms could not be recorded until plants reached maturity. This limited the number of plants which could be tested and thus added to the difficulty of developing resistant varieties. Large numbers of plants could be tested, however, if a suitable seedling test could be developed. It was decided to investigate the possibility that *V. dahliae* would produce a wilt toxin which could be used to differentiate between resistant and susceptible varieties. Bewley (1922) was the first to record the formation of a toxin by the closely related fungus *V. albo atrum*, isolated from tomatoes, so it was considered that a wilt-inducing toxin would also be produced by *V. dahliae*.

The taxonomy of *Verticillium* species is still unsettled, but the authors prefer to follow Isaac (1949) and separate *V. dahliae* from *V. albo atrum*. New Zealand isolates can be quite readily separated morphologically into: (a) those without microsclerotia, having only longitudinally associated carbonised hyphae; and (b) those without

carbonised hyphal threads but with definite irregularly-shaped microsclerotia. Other workers prefer to include *V. dahliae* under *V. albo atrum*, apparently because of the existence, in other countries, of isolates which have both morphological types of resting hyphae (Keyworth 1952).

Experimental

Wilting Trials with Spore Suspensions and Culture Extracts

In preliminary trials with tobacco seedlings, a temporary wilting occurred after dipping roots in a spore suspension of *V. dahliae* and then transplanting them to sterilised soil in pots. These plants soon recovered and grew rapidly. Further subsequent inoculations with 10 million spores per plant at the three-leaf stage again resulted in a temporary wilting of some plants.

Various media, including potato detrose agar, dried peas, and oatmeal all give excellent spore production, and heavy inoculations gave good development of wilt symptoms as the plants reached maturity under high light intensities.

These initial trials with tobacco seedlings were carried out at Lincoln; but later work, using detached stems with leaves, was carried out at Motueka because it was thought that the greater intensity of sunlight and warmer temperatures would provide a more favourable environment for testing. Three varieties, Virginia Gold and Harrison's Special, which had shown susceptibility, and Amarello, which had shown some resistance, all in field trials, were selected. Lengths of stem, with three or four vigorous leaves from previously topped plants of these varieties, were used to test for wilting. A trial had already demonstrated that such sections retained their turgidity for more than two weeks under maximum light conditions in the glasshouse, when the lower ends were placed in water.

The toxin production of various species and isolates of *Verticillium* was tested by placing the cut ends of the stems in spore or culture suspensions of the fungi.

In the first test one isolate each of *V. dahliae* and *V. albo atrum* were grown on sterilised pea seed (Cruickshank 1953) in 250 ml flasks, shaken with distilled water and strained through muslin, and the extract made up to 1,000 ml. For testing, cut stems of 12 plants each of the varieties Harrison's Special, Amarello, and Virginia Gold were placed in two concentrations of the extract, 8.3% and 0.83%, made by diluting with sterilised water. Control plants were placed in distilled water.

Severe wilt symptoms showed in the leaves of Harrison's Special and Virginia Gold after 10 days, and mild symptoms appeared in Amarello leaves after 15 days in the 8.3% extract in the *V. dahliae* series (Table 1). Wilting did not occur in the 0.83% extract, nor in the *V. albo atrum* cultures, nor in the controls, although there was a reduction in transpiration in both the *V. albo atrum* and the 0.83% *V. dahliae* series. These results suggested that, by a screening technique using culture toxins, it would be possible to find tobacco plants field-resistant to Verticillium wilt.

TABLE 1. Wilting and Transpiration of Detached Tobacco Shoots Placed 15 Days in Culture Extracts of *V. dahliae* and *V. albo atrum* (Wilting: 4 = severe; 1 = slight; 0 = nil)

Variety	Field Reaction	*V. dahliae* Extract				*V. albo atrum* Extract				Check	
		8.3%		0.83%		8.3%		0.83%			
		Wilt	Transpiration %	Wilt	Trans. %	Wilt	Trans. %	Wilt	Trans. %	Wilt	Trans. %
Harrison's Special	Susceptible	4	17	0	50	0	50	0	75	0	100
Virginia Gold	Susceptible	4	17	0	50	0	50	0	75	0	100
Amarello	Resistant	1	25	0	50	0	50	0	75	0	100

The next trial was made to compare the toxin production of different isolates of *V. dahliae*. Those chosen were V7 ex *N. tabacum*, Motueka; V20 ex *N. sanderae*, Auckland; and V47 ex *Rubus ideaus*, England. The same methods of culturing and testing as in the previous trial were used and the wilting of Harrison's Special shoots recorded after 15 days. Most severe symptoms were produced by isolate V7, moderate symptoms by V20, and mild symptoms by V47. The amount of transpiration in the inoculated series was again greatly reduced compared with that of the controls. These tests suggested that the isolate from tobacco was a specialised strain adapted to high toxin production as previously found by Christie (1956).

Although this technique of placing cut stems of tobacco was a distinct advance on the field method, it still did not enable large numbers of plants to be screened for resistance. It was therefore decided that large-scale toxin production for use on seedling material should be further investigated.

Toxin Production

Several trials were made to compare different carbon and nitrogen ratios in a synthetic nutrient medium based on Czapek's formulae (KH_2PO_4, 1.0 g; KCl, 0.5 g; $MgSO_4 7H_2O$, 0.5 g; $FeSO_4$, 0.01 g; $NaNO_3$, 2.0 g; sucrose, 30 g; distilled water, 1 l). When a solid media was required 1.5% agar was added. The five different C/N

TABLE 2. Grammes of Nutrient/Litre

Nutrient	Carbon : Nitrogen Ratio				
	H1	H/m	M/m	M/h	L/h
Sucrose	75.0	75.0	30.0	30.0	7.5
$NaNO_3$	1.5	2.0	2.0	5.0	5.0

ratios were obtained by varying the amounts of sucrose and sodium nitrate according to Table 2.

The two isolates of *V. dahliae* were V7 and V20, used previously. Inoculation of media was carried out using a freshly prepared spore suspension containing 200,000–250,000 spores per ml.

Liquid media (used only for the more pathogenic V7 isolate) was incubated at 21–25°c in a shake flask machine using 100 ml to each 500 ml flask.

Agar cultures were grown in petri dishes containing 12–15 ml per plate at 21–25°c, and six replicates were made for each treatment. The incubation period was either five or eight days.

The three substrates for determining toxin production were as follows:—

(1) *Culture suspensions.* Liquid cultures were macerated in a blender for 90 seconds. Agar cultures were macerated (six plates in 100 ml sterile water) for 90 seconds and then strained through muslin.

(2) *Culture filtrates.* These were prepared from unfiltered suspensions by centrifuging at 3,500 r.p.m. for 10 minutes, and then decanting the liquor, which was then filtered under pressure in an "Equipk" bacterial filter.

(3) *Mycelial suspension.* When required, the mycelial residue from the culture filtrate preparations was resuspended in 100 ml of sterile water.

The tests were carried out in a sunny position in a glasshouse, using six leaf seedling plants of Dixie Bright 101, grown in sterilised soil. The roots were well washed before placing in the test liquids held in 4 in. × § in. test tubes. Two or five days after immersion, plants were examined and scored for degree of wilting using the scale: 0 = no wilt, 1 = slight wilt, 2 and 3 = moderate wilt, 4 = severe wilt.

In the first test, using culture suspensions from Czapek's media with different C/N ratios, the cultures were incubated for five days and wilting recorded two days after immersion of the plants in the test liquids. In the second test with culture filtrates and mycelial suspensions, the cultures were incubated eight days and the wilting recorded after five days.

There were no visual differences in mycelial growth or microsclerotial production under shake flask conditions between the different media, although there were differences in conidial production. The more pathogenic isolate, V7, produced conidia more vigorously and microsclerotia less vigorously than the V20 isolate in agar culture. The two extreme C/N ratios, H/1 and L/h. produced least microsclerotia and conidia in agar culture.

The results of wilt development in these two trials are given in Table 3.

These results confirmed previous tests in that toxin production was greater with the V7 isolate and also showed the superiority of

TABLE 3. Wilting of Tobacco Seedlings in *V. dahliae*, Culture Suspensions (CS), Culture Filtrates (CF), and Mycelial Suspensions (MS)

C/N Ratio	*V. dahliae* ex Tobacco V7						*V. dahliae* ex N.spp V20			Total
	Liquid Culture			Agar Culture			Agar Culture			
	CS	CF	MS	CS	CF	MS	CS	CF	MS	
H/l	5	12	5	8	2	1	2	1	1	37
H/m	8	12	5	8	4	0	4	3	0	44
M/m	7	9	—	9	0	—	6	0	—	31
M/h	10	8	0	10	0	0	6	0	0	34
L/h	10	3	0	7	0	0	3	0	0	23
Check	0	0	0	0	0	0	0	0	0	0

liquid (shake flask) culture. In addition, they showed that the toxin was largely associated with the filtrate rather than the mycelium, and suggested that the best medium for toxin production was that with the H/m C/N ratio.

Another trial was carried out to find if the toxin was heat-stable, and how dilution affected the wilting ability of the toxin. The range of dilutions was: full strength, 0.1, 0.01, 0.05, and 0.001. The heat stability was tested by autoclaving at 15 lb pressure for 15 minutes. The results, given in Table 4, showed that dilution and autoclaving reduced the wilting ability of the culture filtrate.

TABLE 4. Effect of Dilution and Heat on the Wilting Ability of Culture Filtrates of *V. dahliae* (V7 isolate)

Dilution	Liquid Culture	Agar Culture	Total
1.0	6	6	12
0.1	5	1	6
0.01	3	3	6
0.05	1	2	3
0.001	1	2	3
Autoclaved 1.0	3	4	7
Check	0	0	0

Discussion

The relatively small amount of mycelial suspension inoculum which was added to the water in which the cut stems were placed, and the rapidity with which transpiration, as measured by decrease in liquid level, was halted, suggested that wilting in tobacco was due to a toxin

produced by the fungus rather than by mechanical plugging of the vascular elements by mycelium. This was further supported by the fact that culture filtrates resulted in more severe wilting than mycelial suspensions.

In all these tests the intensity of light appeared to have a marked effect on the severity of wilt in tobacco seedlings. In one test, although the first day was overcast, the second day was bright and sunny, and plants in the treatment liquids wilted rapidly. During later tests five cloudy days occurred and it was not until the fifth day that severe wilt occurred in any plants.

These results showed that it should be possible to evolve a satisfactory technique for screening large numbers of tobacco seedlings for resistance to Verticillium wilt by testing them in sunlight using culture filtrates from a pathogenic strain of *V. dahliae* grown in shake flask culture on a suitable medium. The best medium tested was Czapek's, modified by increasing the sucrose from 0.03% to 0.075%.

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INSECTS ASSOCIATED WITH THE MAJOR FODDER CROPS IN THE NORTH ISLAND

III. COLEOPTERA*

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Summary

A survey during 1959 of fodder crops in the North Island revealed some 42 species of Coleoptera. Their identity, distribution, incidence, and possible economic significance are discussed.

Introduction

A survey of the insects associated with fodder crops in the North Island was carried out during the summer of 1959. A general account of this survey (Eyles 1960a), and the information in respect of the Hemiptera (Eyles 1960b) have already been presented.

The present paper, which concerns the Coleoptera collected in the survey, gives information on their distribution, incidence, and possible economic importance. Distribution maps for two species are presented, a black square indicating at least one occurrence within that area.

About two dozen (including one useful) species of Coleoptera are recorded in the New Zealand literature as occurring on fodder crops. Marshall (1931) mentions two species of *Catoptes* on turnips and a further one on mangels. Cottier (1932) mentions 18 species on swedes.

Results

Suborder **ADEPHAGA**

Fam. CICINDELIDAE (Tiger Beetles)

Adults and larvae in this family are predaceous and occur usually in dry areas.

* Where further information is contained in Cumber's pasture accounts (1959, 1960), it is indicated by an asterisk.

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Co. 18 *Cicindela tuberculata* F.*

This species is known to occur throughout the North Island. A single specimen was taken from turnips at Te Teko, 12.2.59. It is predaceous, perhaps playing a very minor beneficial role.

Fam. CARABIDAE (Ground Beetles)

The family includes mainly predaceous species.

Co. 43 *Clivina rugithorax* Putz.*

This native species is distributed throughout the North Island. In the present survey single adults were taken in the soil from lucerne, Bulls (16.3.59); turnips, Marumaru (10.2.59); and swedes, Marton (24.3.59).

This species usually frequents wet places, being commonly found under logs and stones, and in dried water courses, etc.

Some 20 years ago it was recorded from Pakowhai, Hawkes Bay (Anon, 1938, Muggeridge 1939), as damaging maize kernels and preventing germination of up to 75% of the seed. The numbers taken in the present survey indicate that it is not of general importance.

Fig. 1.—Distribution map for the lathridiid, *Lathridius dualis*. Co. 27.

Co. 57 *Clivina* sp.*

A single adult was taken from the soil in mixed chou and swedes at Maungaturoto on 26.2.59. Three specimens were taken by Mr J. I. Townsend (pers. comm.) at Parere from paspalum-dominant pasture on 28.3.58. Thus it seems to be restricted to Northland.

The habits of the species are not known, but are likely to be similar to those of *C. rugithorax* which it resembles except in its reddening of the anterior portions of the elytra.

Co. 58. *Hypharpax abstrusus* Bates.

This species is known to occur in the north of the South Island. It was taken in small numbers from the soil underneath lucerne at Takapau and Waipawa on 9.2.59. It may play a minor beneficial role.

Co. 42. *Rhytisternus miser* Chaud.

Single specimens were taken from maize at Whangarei (26.2.59) and turnips at Marumaru (10.2.59).

Fig. 2.—Distribution map for the lathridiid *Melanophthalma gibbosa* Co. 1.

Co. 50. *Scopodes elaphroides* White

Members of this genus are small swamp-hunting beetles (Tillyard 1926). *S. elaphroides* is one of our commonest and most widespread species (Hudson 1934). In the present survey a single specimen was taken from turnips at Maungaturoto (26.2.59). It is unlikely to be of importance in fodder crops.

Co. 63. *Scopodes multipunctatus* Bates

This species is recorded from Auckland (Broun 1880). A single specimen was taken from turnips at Marton on 24.3.59.

Co. 59. Undetermined ***

A single specimen was taken from maize at Dargaville on 26.2.59.

Suborder **POLYPHAGA**

Fam. SILPHIDE

Nearly all species in this family feed on carion, but the larvae of some are predaceous on snails and lepidopterous larvae, and some species are vegetarian and damage root crops (Imms 1957).

Co. 41. Undetermined

This species (tribe Agyrtodini) belongs either to the genus *Zeagyrtes* or to *Zearagytodes* (Mr E. S. Gourlay pers. comm.). Two specimens were taken from turnips at Te Teko on 12.2.59.

Fam. STAPHYLINIDAE (Rove Beetles)

Many members of this family are predaceous, but the majority live on decaying organic matter, e.g., dung and dead animals.

Co. 14. *Atheta* sp.*

This species is widely distributed in the North Island. It was taken in small numbers on the following crops: lucerne at Greytown (8.4.59); maize, Te Kaha (11.2.59), Dargaville (26.2.59); mixed chou and swedes, Levin (25.3.59); Turnips, Te Kuiti (20.3.59), Te Arakura (24.3.59), Otaki (25.3.59); and swedes, Pahiatua (8.4.59).

The species is probably associated with dead leaf and damaged plants. No detailed information on its habits is available, so its importance cannot be gauged.

Co. 55. *Conosomus* (near) *brevicornis* Broun

A single specimen was taken from a mixed chou and swede crop at Otorohanga on 19.3.59.

Co. 47. *Creophalus oculatus* (F.)

This species occurs throughout New Zealand. A single specimen was taken from chou at Whakatane on 12.2.59. It is a carion feeder and not important in crops.

Co. 54. Undetermined

A single specimen was taken from lucerne at Te Awamutu on 19.3.59.

*** Undetermined species are held under the numbers given in the collections of the Entomology Division, Palmerston North.

Fam. SCARABAEIDAE (Chafers)

Members of this family have typical curved larvae which live in the soil among roots, or in decaying wood, leaf litter, and other rotting vegetation. Adults of many species are nocturnal or crepuscular, but some are diurnal.

Co. 62. *Costelytra* (*Odontria*) *zealandica* (White) (Grass-grub)

This species occurs throughout New Zealand. In the present survey larvae were taken from the soil as follows: lucerne at Waipapa (24.2.59), Cambridge (3.3.59), Waiotapu (12.2.59), Waipawa (9.2.59), and Greytown (8.4.59); maize at Pakaraka (24.2.59); mixed chou and swedes at Irirangi (5.3.59) and Inglewood (16.3.59); and swedes at Waitara (16.3.59). The greatest number (38) was taken from lucerne at Waiotapu.

The habits and life cycle are well known (Kelsey 1951 a, b). This species is one of the most destructive pasture pests in New Zealand. Damage is also reported from forest nurseries (Miller 1945), and in carrots (Kelsey 1951a, b). Kelsey also records damage to seedling crucifers, and defoliation, especially of stone-fruit trees.

The adult stage does not occur during the period of the present survey. Cruciferous crops seemed free from the grub attack, but lucerne stands were frequently infested, the grubs in some cases being associated with the grasses which inevitably are present in older stands.

Fam. HELODIDAE

These insects have acquatic larvae and adults occur near water.

Co. 8. *Cyphon* sp.*

Cumber (1959) records this species from Kaitaia, and it is also present at Te Kuiti (24.1.57). In the present survey it was taken in small numbers from maize at Shannon (25.3.59), chou at Mercer (3.3.59) and Kihikihi (19.3.59), and turnips at Te Kuiti (20.3.59). It is thus apparently widely distributed in the North Island. It is not important in fodder crops.

Fam. ELATERIDAE (Click Beetles)

The larvae (wireworms) of many species are extremely destructive to pastures, cereals, and root crops.

Co. 33. *Lacon variabilis* Cand.*

This introduced species is now widespread. Two adults and 12 larvae were taken from lucerne at Waipawa (9.2.59), and a single adult from maize at Dargaville (26.2.59). A small number of larvae, probably belonging to this species was taken from turnips at Tiniroto (10.2.59) and Marton (24.3.59). Larvae were probably responsible for some of the damage evident in the lucerne at Waipawa, but the situation was complicated by the heavy populations of the white fringed weevil larvae which were present.

Co. 48. *Monocrepidius exsul* Sharp

This introduced species is present throughout the North Island (Hudson 1934). One adult was taken from swedes (soil) at Waimungu on 12.2.59. Hudson indicates that the larvae are common in gardens and that the beetles appear at dusk. There is no indication that this species is a pest of fodder crops.

Fam. NITIDULIDAE

Many species of this family inhabit flowers, others are found on fungi or in decaying animal matter, while some are known to damage crucifers (Imms 1957).

Co. 49. *Ips asper* (Broun)

A single specimen was taken from maize at Kaitaia on 23.2.59.

Fam. CUCUJIDAE

Members of this family are mainly predatory, but grain-infesting species are known (Imms 1957).

Co. 44. *Cryptamorpha suturalis* White

This appears to occur mainly in the northern portion of the Island (Broun 1880), with Tairua as the southernmost recorded locality. In the present survey a single specimen was taken from maize at Whirinaki on 25.2.59. This species is unlikely to prove of importance in fodder crops.

Fam. CRYPTOPHAGIDAE

The adults of this family feed on fungi or live in flowers, whilst the larvae are mainly scavengers of straw, refuse, bee and wasp nests, and gregarious caterpillars (Tillyard 1926).

Co. 26. *Micrambina helmsi* Reitt.

This species occurs in both North and South Islands. Four specimens were collected from maize at Whangarei on 26.2.59, and one from mixed chou and swedes at Foxton on 25.3.59. It is not important in fodder crops.

Co. 60. Undetermined

Single specimens were collected from maize at Whangarei (26.2.59) and Ohinewai (3.3.59).

Fam. CORYLOPHIDAE

These small beetles are usually associated with decaying wood or vegetation.

Co. 4. *Sericoderus* sp.*

This species is widespread in the North Island south of Auckland. It occurred in small numbers on lucerne at Greytown (8.4.59), maize at Ohinewai (3.3.59), chou at Carterton-Masterton (8.4.59) and Ohinewai (3.3.59), and turnips at Silverdale (2.3.59) and Otaki (25.3.59). It is of no importance on fodder crops.

Fam. COCCINELLIDAE (Lady-bird Beetles)

Most coccinellid beetles and larvae are predaceous and many are highly beneficial species.

Co. 56. *Adalia bipunctata* (L.)

A single adult was taken from mixed chou and swedes at Konini on 8.4.59. It was possibly from the associated sowthistle which harboured many aphids. This is a beneficial species.

Co. 10. *Coccinella 11-punctata* L.*

This cosmopolitan species is widespread in the North Island. Some 20 adults and 10 larvae were taken from lucerne at Waipawa (9.2.59), Waiotapu (12.2.59), and Greytown (8.4.59); chou plus green-feed barley, Martinborough-Greytown (8.4.59); turnips, Te Teko (12.2.59), Rangiriri (3.3.59), and Te Puke (4.3.59); and swedes, Marton (24.3.59) and Pirinoa (8.4.59). This well-known species is highly beneficial because of its aphid predation.

Co. 9. *Scymus* sp.*

This species is widely distributed in the North Island. In the present survey it was taken in small numbers from maize at Hangaroa (10.2.59) and Whirinaki (25.2.59), chou at Mercer (3.3.59), mixed chou and swedes at Mokau (16.3.59), and turnips at Puketona (24.2.59) and Te Puke (4.3.59). More than one species is possibly involved here.

Fam. LATHRIDIIDAE

These small beetles are mainly fungus feeders.

Co. 27. *Lathridius dualis* Broun

In describing this species, Broun (1914) records it from Ardagh, McClennan's Bush, and Methven. The distribution encountered in the present survey is shown in Fig. 1. Although widespread, it is of more common occurrence in the southern portion of the Island. Twenty-four adults were taken from 16 crops during Febuary, March, and April, which included lucerne, maize, chou, swedes, and turnips. The species is not considered important, although it could possibly be implicated in the spread of fungus diseases.

Co. 1. *Melanophthalma gibbosa* Herbst.*

This cosmopolitan species occurs throughout the North Island. The distribution encountered in the present survey is shown in Fig. 2.

This species occurred on all crops studied except pampas. Some 1,400 adults were taken from lucerne (11 crops), maize (28), chou (13), chou plus swedes (12), turnips (19), swedes (5), mangels (4), rape (1), and a mixture of rape, swedes, and turnips (2). The greatest catch was 93 on turnips at Marton (24.3.59), but it often occurred in high numbers (i.e., over 30) on maize, turnip, and swede crops.

The insect occurred in far greater numbers in fodder crops than in pastures. This may be a reflection of the shelter afforded by the

larger leaves of crops, or of the greater moisture and humidity in crops producing more fungal growth on which the insects feed. This is the most common coleopteron occurring on fodder crops. It is not regarded as important, however, although it could play a minor part in fungus dissemination.

Co. 36. *Melanophthalma* sp.

Single specimens were taken from lucerne at Greytown on 8.4.59, and turnips at Makerua on 25.3.59.

Co. 61. *Melanophthalma* sp.

Small numbers of this species were collected from lucerne at Martinborough and Greytown, chou plus greenfeed barley at Martinborough-Greytown, turnips at Greytown, and swedes at Pirinoa, all on 8.4.59. This species, which is possibly a variant of *M. gibbosa*, may be restricted to the Wairarapa.

Fam. MYCETOPHAGIDAE

Most species in the family are associated with fungi.

Co. 37. *Triphyllus hispidellus* Broun

A single adult was collected from chou at Carterton-Masterton on 8.4.59.

Co. 6. *Triphyllus serratus* Broun

A single adult was taken from maize at Helensville on 2.3.59.

Fam. TENEBRIONIDAE

Habits are variable within this family. Many species occur on the ground, many are wood-eating, some live in grain and other stored foods, the larvae being known as "meal-worms", while others live in dung, dead animals, or fungi (Imms 1957).

Co. 39. Undetermined

A single adult was taken from lucerne (soil) at Takapau on 9.2.59.

Fam. CHRYSOMELIDAE

The members of this very extensive family are phytophagous or xylophagous species and include many agricultural pests.

Co. 5. *Inopelonia testacea* Broun*

This species occurs throughout the North Island. Small numbers were collected from lucerne at Waipapa (24.2.59) and Bulls (16.3.59), maize at Pakaraka (24.2.59) and Waipawa (9.2.59), chou at Mercer (3.3.59) and Urenui (16.3.59), turnips at Te Kuiti (20.3.59), and swedes at Waitara (16.3.59). There is no evidence that it is of economic importance in fodder crops.

Co. 45. *Phyllotreta paspalae* Broun

Single specimens were collected from maize at Eureka (3.3.59) and turnips at Ruakaka (2.3.59).

Co. 52. *Phyllotreta vittigera* Broun
A single specimen was taken from turnips at Rangiriri on 3.3.59.

Fam. APIONIDAE

Insects in this family are often associated with seeds, roots, or stems, and some are injurious to leguminous crops.

Co. 3. *Apion ulicis* Forst.*

This European species is now well distributed in New Zealand. In the present survey a single adult was taken from lucerne at Martinborough on 8.4.59, but no gorse was in the immediate vicinity. Cumber (1959) also records this species, apparently in the absence of its considered host.

Fam. CURCULIONIDAE (Weevils)

This family contains a great number of injurious plant feeders.

Co. 51. *Acalles* (near) *furvus* Broun

A single specimen was collected from maize at Whangarei on 26.2.59.

Co. 7. *Catoptes obliquisignatus* Bohem.*

This native species is widely distributed in the North Island. Single specimens were taken from lucerne at Taumarunui (20.3.59), maize at Longburn (24.3.59), and mixed chou and swedes, and mangels, both from Kimbolton (24.3.59). Larvae occurred in the soil underneath lucerne at Waipapa (24.2.59).

Three *Catoptes* spp. are reported by Marshall (1931) as damaging turnips and mangels in New Zealand. In the present survey there has been no indication that it is a serious pest of fodder crops.

Co. 53. *Desiantha maculata* Blackburn

This is an introduced species from South Australia. The A. E. Brookes collection contains one specimen from Nelson (1914), and another from Riverhead (October 1930) (Mrs B. M. May pers. comm.). In the present survey single specimens were collected from lucerne at Waipawa (9.2.59) and turnips at Te Puke (4.3.59). In testing sampling techniques a further specimen was taken from chou at Palmerston North in late January.

Co. 40. *Graphognathus leucoloma* (Boheman) (White-fringed Weevil)*

Three species of *Graphognathus* occur in South America and the United States. These and their several races are similar in appearance and habits (Roberts 1952). It has recently been reported from Australia (Metcalf *et al.* 1951). Discovered in North Auckland about 1944, it is believed to have been introduced into Auckland about 1940 with U.S. war equipment. It has gradually spread, and in the last five years has become a pest in the Helensville and Warkworth districts. In 1957 it appeared as a pest in Hawkes Bay where it is now known to occur fairly widely. Sixty-five larvae and 2 pupae were taken in the soil from a lucerne sampling at Waipawa on 9.2.59. This represents

approximately 20 larvae per square foot. At a later investigation (26.3.59) one live and two dead adults were taken, and many larvae but no pupae were found.

The larvae feed on plant roots, and adults, which are all females and cannot fly, do some damage by feeding on foliage. At Waipawa the majority of the larvae were in their final instar and they occurred mainly in the area immediately underneath the crowns of plants.

Roberts (1952) states that the larvae may damage many plants, e.g., cotton, maize, peanuts, velvet beans, clover, alfalfa, field crops, garden and ornamental plants, and weeds. The infested lucerne crop referred to above was patchy, with gaps between plants. Affected plants wilted and turned yellow before dying.

The plants at times seriously affected in New Zealand include pasture species, exotic forest seedlings, lucerne, peas, and potatoes. This insect shows possibilities of becoming a major pest.

Co. 11. *Hyperodes bonariensis* Kushel* (Argentine Stem Weevil)

This cosmopolitan species, previously referred to as *H. griseus*, occurs in both North and South Islands from sea-level to several thousand feet. It was taken in widely separated North Island localities in pastures (Cumber 1959).

In the present survey, small numbers were taken from lucerne at Greytown (8.4.59) and maize at Shannon (25.3.59).

The habits have been noted in studies on wheat (Morrison 1938) and rye-grass (Kelsey 1958). The majority of the damage in both cases was caused by the larvae which tunnel in the plant tillers. It appears to be a more serious pest of rye-grass than of wheat. Kelsey records adults as feeding on all grasses except Yorkshire fog, on clovers, lucerne, cereals, and cruciferous crops, and the larvae on rape in the South Island. Lowe (1958) records damage to crucifer seedlings.

Co. 38. *Peristoreus sexmaculatus* (Broun)

Two specimens were collected from maize at Shannon on 25.3.59.

Discussion

In surveys of the present type, it is to be anticipated that a large number of unimportant species will occur in low numbers. It is, of course, possible that species occasionally or locally important will have been missed or will have occurred only in small numbers. The carabid *Clivina* spp. may belong here. The species which are most likely to be damaging to short rotation crops are those which frequent pastures and which, owing to insufficient mechanical preparation of the soil, remain in numbers to attack the crops which follow. In perennial crops such as lucerne, the situation is different, and pests may build up relatively undisturbed.

The present survey has not indicated any new and unsuspected damaging species, but it does confirm that the white-fringed weevil *Graphognathus leucoloma* promises to be a very considerable problem in crops such as lucerne, or where adequate mechanical and grub-freeing operations are not undertaken between crops.

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APPENDIX

SPECIES OF COLEOPTERA ASSOCIATED WITH FODDER CROPS

	Lucerne	Maize	Chou Moellier	Chou Moellier, Swedes, Mixed	Turnips	Swedes	Mangels	Rape	Rape, Swedes, Turnips, Mixed
Cicindela tuberculata					*				
Clivina rugithorax	*				*	*			
Clivina sp. Co. 57				*					
Hypharpax abstrusus	*								
Rhytisternus miser		*			*				
Scopodes elaphroides					*				
Scopodes multipunctatus					*				
undetermined carabid Co. 59		*							
Atheta sp. Co. 14	*	*		*	*	*			
Conosomus near *brevicornis*				*					
Creophalus oculatus			*						
undetermined staphylinid Co. 41					*				
undetermined staphylinid Co. 54	*								
Costelytra zealandica	*	*		*		*			
Cyphon sp. Co. 8		*	*		*				
Lacon variabilis	*	*							
Monocrepidius exsul						*			
Ips asper		*							
Cryptamorpha suturalis		*							
Micrambina helmsii		*		*					
undertermined cryptophagid Co. 60		*							
Sericoderus sp. Co. 4	*	*	*		*				
Adalia bipunctata				*					
Coccinella 11-punctata	*		*		*	*			
Scymus sp. Co. 9		*	*	*	*				
Lathridius dualis	*	*	*	*	*	*			
Melanopthalma gibbosa	*	*	*	*	*	*	*	*	*
Melanopthalma sp. Co. 36	*				*				
Melanopthalma sp. Co. 61	*		*		*	*			
Triphyllus hispidellus			*						
Triphyllus serratus		*							
undetermined tenebrionid Co. 39	*								
Inopelonia testacea	*	*	*		*	*			
Phyllotreta paspalae		*			*				
Phyllotreta vittigera					*				
Apion ulicis	*								
Acalles near *furvus*		*							
Catoptes obliquisignatus	*	*		*			*		
Desiantha maculata	*		*		*				
Graphognathus leucoloma	*								
Hyperodes griseus	*	*	*						
Peristoreus sexmaculatus		*							

PATHOLOGICAL EFFECTS OF FEEDING *Pithomyces chartarum* (Berk. & Curt.) M. B. Ellis TO GUINEA-PIGS

By M. M. MacKinnon* and W. A. Te Punga, Wallaceville Animal Research Station, Department of Agriculture, Wellington

(*Received 2 November 1960*)

Summary

A grading system for biliary epithelial hyperplasia, damage to the interlobular bile ducts, vascular lesions, and focal coagulative parenchymal necrosis caused by feeding *Pithomyces chartarum* to guinea-pigs, has been evolved.

Introduction

Thornton and Percival (1959) reported that a fungus identified as *Pithomyces chartarum* (Berk. & Curt.) M. B. Ellis (formerly *Sporidesmium bakeri* Syd.) caused lesions in sheep and guinea-pigs similar to those caused by feeding grass collected during an outbreak of facial eczema. This discovery has opened much broader avenues of investigation. Some of the biological aspects of this work require quantitative estimates of damage produced by this fungus in test animals. At the same time it is desirable that the specificity of reaction should be confirmed.

Lesions in guinea-pigs resulting from feeding toxic grass have been described by Evans *et al.* (1957) and Perrin (1957). The latter distinguished three grades of lesions in guinea-pigs which survived the test period of four weeks. The association of the fungus *P. chartarum* with facial eczema and the consequent availability of pure fungal cultures made it possible to re-examine the guinea-pig as a test animal.

Preliminary investigations were designed: (a) to confirm that the lesions found were consistent with those caused by feeding toxic grass; and (b) to investigate pathological changes which might be correlated with the amount of fungal material administered.

Materials and Methods

Previous investigations using the guinea-pig as a test animal resulted in the following conditions being adopted in this study.

Guinea-pigs weighing 120 ± 3 g were taken from the colony. They invariably lost weight for 24 to 48 hours, and those failing to regain their original weight by the third day were rejected. For the

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duration of any test procedure guinea-pigs occupied individual cages. The animals were fed the standard pelleted ration used at the Wallaceville Animal Research Station. This was supplemented by green feed (silver beet). Water was supplied *ad lib.*

P. chartarum cultures were kindly supplied by the Soil Bureau, Department of Scientific and Industrial Research, Taita. Mycelium and spores were separated from the culture fluid, homogenised with distilled water in a one gallon Waring Blendor, divided into quantities sufficient for a day's dosing, and stored at —15°c until required. On the day of feeding, samples were rapidly thawed in a 37°c water bath.

Fungal material was administered from a hypodermic syringe fitted with an 18 gauge needle which was inserted into 5 cm of polyethylene tubing with an outside diameter of 2 mm. Fungal homogenates were well mixed before being drawn into the syringe. The tip of the polyethylene tube was inserted into the guinea-pig's mouth and a small portion of the dose administered. Ample time for swallowing each portion was allowed before continuing the dosing. By this method doses of up to 5 ml could be administered within three minutes.

Groups, each of two guinea-pigs, were given a daily dose of 1, 2, 3, 4, or 5 ml for 7, 14, 21, or 28 days. Animals on each dose level were killed at 7, 14, 21, 28, or 35 days. In addition, undosed control animals were killed at similar intervals.

Each guinea-pig was examined on the day of death or slaughter for evidence of jaundice or signs of any complicating disease condition. The examiner was given no indication of prior treatment of the guinea-pigs. The livers were removed and examined macroscopically for colour, shape, size, degree of pliability, and presence of universal or focal lesions. At this stage an attempt was made to grade any liver damage found according to the method of Perrin (1957).

Each liver was then immersed in 10% formalin for at least 48 hours. Multiple samples, taken from each liver after fixation, were submitted to routine histological techniques using Delafield haematoxylin and eosin.

Results

(1) Macroscopic Lesions

No evidence of concurrent complicating disease was found. No jaundice was noticed, but some of the carcasses examined several hours after death showed greenish discoloration of subcutaneous tissues.

Abnormalities were recorded from most of the livers examined, including those from animals which were dosed for seven days only. These varied from mild changes indistinguishable from those seen in some of the control guinea-pigs to obvious changes definitely attributable to the ingestion of fungus. The latter were readily differentiated into chronic progressive, and acute focal lesions. Both types of lesion were found simultaneously and separately.

No specific lesions were detectable until 7–10 days after the first dose; but, once established, they developed more or less rapidly even after dosing was stopped. A compensatory response in the form of nodular hyperplasia was seen in some severely affected livers.

Chronic Progressive Lesions. In the early stages of development these lesions occurred focally as more or less circumscribed areas on lobes, in zonal distribution on the borders, or involving a considerable area, usually the dependant portion, with a clear line of demarcation between affected and unaffected tissue. Many livers, however, appeared to be uniformly involved from the outset. At later stages the focal lesions tended to become lobar in distribution, finally involving the whole liver. Occasionally there was uniform involvement of all lobes except one.

Although the livers of control animals varied somewhat in colour, related to the degree of bleeding out, severely damaged livers were pale in colour, tinged with brown or olive green.

Livers showing well developed lesions were obviously swollen, frequently had rounded edges, a distinctly rubbery texture, and a roughened capsule due to minute pitting of the surface.

Exaggeration of the lobular pattern was most obvious in cases of moderate damage. In advanced cases lobular pattern was obliterated, with an appearance of gross fibrosis. Focal occurrence in mild cases could be regarded as evidence of specific damage.

Necrosis was not always present. When observed, it was often associated with exaggerated lobular pattern occuring as either minute foci close together, giving a fine stippled appearance, or as larger, irregular, pale foci up to 2 mm in diameter.

Acute Focal Lesions. These were infarct-like lesions characterised by an irregular polygonal shape and sharply defined boundaries, varying in size from a few millimeters to areas involving the greater part of a lobe. Usually these lesions were situated on the border of a lobe. They were pale, stained with green or yellow, and occasionally surrounded by a dark red border.

(2) Microscopic Lesions

A remarkable uniformity was found to occur in the distribution of microscopic lesions in all lobes of the liver of each guinea-pig. The damage could be divided into four main types: (a) biliary epithelial hyperplasia (so-called bile duct proliferation); (b) vascular lesions; (c) damage to the interlobular bile ductules; and (d) focal parenchymal coagulative necrosis. These lesions, considered singly or collectively, formed the basis of the following grading system for quantitative assessment of liver damage:—

(a) *Biliary Epithelial Hyperplasia*

Grade 1. Evidence of early proliferative change in biliary epithelium as shown by increased size of, and irregularity in, the

epithelial cells in some interlobular bile ductules and also in a few intralobular radicles (canals of Herring) and intralobular ductules following the course of intralobular vascular radicles either towards the central vein or towards a neighbouring portal tract area.

Grade 2 Signs of actual bile duct proliferation within a few portal tracts evidenced by increased numbers and prominence of small interlobular bile ductules. Increased hyperplasia in intralobular radicles, not yet recognisable as pseudo-lobulation.

Grade 3. Grade 2 changes in interlobular bile ductules in the majority of portal tracts and early pseudo-lobulation.

Grade 4. Biliary epithelial hyperplasia in short intralobular radicles almost completely surrounding portal tracts and encroaching slightly upon the lobular parenchyma. Obvious pseudo-lobulation along the course of the longer intralobular radicles.

Grade 5. Biliary epithelial hyperplasia becoming gross and encroaching upon the lobules, with marked loss of normal parenchyma.

Grade 6. Complete overwhelming of the lobular parenchyma by biliary epithelial hyperplasia, with only narrow strips and islands of normal parenchyma remaining.

(b) *Vascular Lesions*

Grade 1. Mild degenerative changes, evidenced by hyaline appearance of small portal vein walls and hydropic change in the walls of medium-sized portal veins.

Grade 2. Evidence of atrophy of walls of medium-sized portal veins characterised by thin, ragged wall structure. Subintimal oedema and mild lymphocytic infiltration in some smaller branches.

Grade 3. Some focal necrosis in portal blood vessel walls.

Grade 4. Extensive necrosis in portal blood vessel walls.

Grade 5. Phlebitis and/or thrombosis in portal veins.

(c) *Damage to the Interlobular Bile Ductules*

Grade 1. Hypertrophy, ballooning, and loss of staining affinity of the epithelial cells of interlobular bile ductules.

Grade 2. Epithelial hyperplasia in interlobular bile ductules, or metaplasia in distended ductules, with low cuboidal or pavement forms in their lining epithelial cells.

Grade 3. Changes of Grade 2 plus necrosis of epithelial cells.

Grade 4. Well established necrosis in interlobular bile ductules with commencing extension into the surrounding portal tract.

Grade 5. As for Grade 4 plus a surrounding inflammatory reaction frequently involving the adjacent branch of the portal vein causing thrombo-phlebitis.

Grade 6. Massive necrosis centred upon the remains of an interlobular bile ductule involving all tissue to a considerable depth, often including lobular parenchyma but showing no surrounding inflammatory reaction.

(d) *Focal Parenchymal Coagulative Necrosis*

These lesions were classified in degrees of: (i) frequency of observation; and (ii) extent of individual lesions:—

(i) *Frequency of Observation*

Degree 1—less than seven times per sq. cm. section.
Degree 2—in many fields per section.
Degree 3—in consecutive fields.

(ii) *Extent of Individual Lesions*

Grade 1—affecting part of a lobule.
Grade 2—affecting a whole lobule.
Grade 3—affecting a whole lobule and part of contiguous lobules.

Discussion

The lesions observed in many of the livers confirmed that damage was similar to that described in the guinea-pig by Evans *et al.* (1957) and Perrin (1957), although some differences of observation or interpretation were noted. It is possible that this could be due to the differences in the basic ration and dosing schedules. Because of these differences, no strict comparison can be made with the degrees of damage distinguished by Perrin (1957).

Macroscopic assessment of liver damage was found to be linked almost entirely with the superficially visible degrees of exaggeration of the normal lobular pattern, which in turn were found to be the macroscopic appearance of biliary epithelial hyperplasia. This method gave a reasonable guide to the advanced degrees of biliary epithelial hyperplasia only. It was difficult, if not impossible, to differentiate mild degrees of specific damage from similar apparently normal variation in the macroscopic appearance of the guinea-pig liver. Greater degrees of specific damage show well-advanced lesions microscopically. In addition, there was a lack of clear-cut landmarks on which to judge degrees of damage macroscopically.

Visible necrosis, including the acute focal lesions, occurred sporadically and not only in guinea-pigs on the higher levels of intake. For this reason it was not possible to correlate the presence or extent of these lesions with dosage.

Microscopic assessment, on the other hand, showed considerable promise. An encouraging feature was the uniformity in distribution of microscopic lesions from all lobes of the liver in individual animals. It is possible that a small number of sections per liver might be adequate for routine examination if the guinea-pig was used as a test animal.

Although a few guinea-pigs died within the first week, no recognisable change was detected except in those which survived to the seventh day. It was not determined which, if any, of the four main types of lesions appeared first. The impression was gained that the first signs of biliary epithelial hyperplasia sometimes coincided with mild degenerative changes in portal veins, and invariably with marked distension of the lymphatic spaces of the portal tracts.

The system of dosing was cumbersome but it ensured uniformity of dosage which should facilitate interpretation of results. On the other hand, since dosing for seven days produced observable lesions, this method of administration is recommended. Dosing on a single occasion would be advantageous but was impractical with our preparations.

It must be emphasised that this is a preliminary report and that gradings are based on a small number of animals only. Further work is envisaged to test these gradings and their correlation with dosage.

Acknowledgments

We should like to thank Drs R. H. Thornton and D. J. Ross of the Soil Bureau, Department of Scientific and Industrial Research, Taita, for supplies of the fungus; Messrs W. Joyce, C. Fenwick, and F. K. Calverley, and Misses E. Sheridan, E. O'Connell, and Mrs C. Van der Burg, for the onerous work of dosing and caring for the experimental animals.

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TOXICITY TESTING OF *Pithomyces chartarum* (Berk. & Curt.) M. B. Ellis IN GUINEA-PIGS

By W. A. Te Punga and M. M. MacKinnon,* Wallaceville Animal Research Station, Department of Agriculture, Wellington

(*Received 2 November 1960*)

Summary

The value of microscopic examination as a quantitative indicator of toxicity of *Pithomyces chartarum* has been studied.

There is good correlation between dose and response when animals are dosed for seven days and examined 21 days from the commencement of dosing.

It is suggested that this procedure could be utilised for biological aspects of the study of *P. chartarum*.

Introduction

Preliminary investigations of the liver damage caused by feeding *Pithomyces chartarum* (Berk. & Curt.) M. B. Ellis (formerly *Sporidesmium bakeri* Syd.) to guinea-pigs (MacKinnon and Te Punga 1961) suggested that grades of damage could be established by microscopic examination. Investigations were extended to assess the potential value of microscopic examinations as a quantitative indicator of toxicity.

Materials and Methods

Guinea-pigs were selected and treated as reported by MacKinnon and Te Punga (1961). They were drenched with fungal material for seven days only, since this period had been shown to be sufficiently long to produce lesions at the dose rates employed.

Livers were treated as reported by MacKinnon and Te Punga (1961), but only three sections were cut from standard sites from each fixed liver (Figs. 1 and 2).

All assessments were made without prior knowledge of the treatment to which an individual animal had been subjected. Numerical gradings of 1 – 6 were given for biliary epithelial hyperplasia and damage to the interlobular bile ductules. Lesions were considered specific if both the above lesions were present together with either vascular lesions or necrosis.

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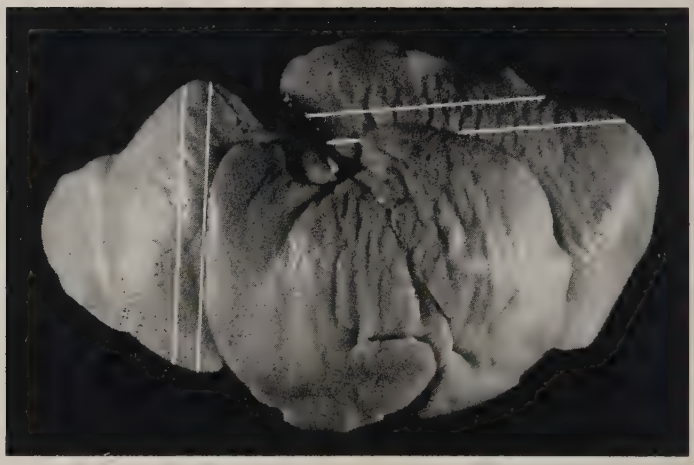

Fig. 1.—Guinea-pig liver showing sites of sections taken for microscopic examination. Diaphragmatic surface. Sections are taken from: (a) right central lobe, parallel to its long axis, from dorsal to ventral borders; (b) left lateral lobe, from dorsal to ventro-lateral border, diagonal to long axis.

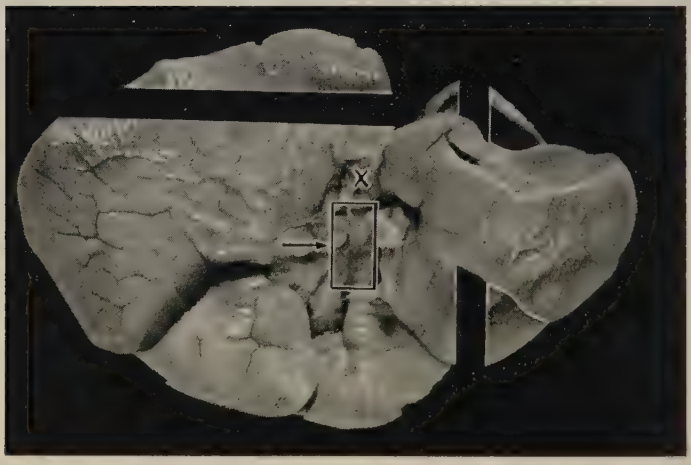

Fig. 2.—Guinea-pig liver showing sites of sections taken for microscopic examination. Visceral surface. The third section is taken from the central lobe and includes the union of the cystic duct, left lobar duct, and bile duct. Arrow indicates where section is presented for cutting. X indicates position of small accessory lobes which have been removed.

Experimental Design

Groups of 4 guinea-pigs were treated with 0, 1, 3, and 5 ml of *P. chartarum* culture per day for 7 days and killed at 14, 21, 28, and 35 days after the first dose.

Results

Results of examinations are shown in Tables 1 and 2.

TABLE 1. Degree of Biliary Epithelial Hyperplasia

Group No.	Dose (ml)	Time of Reading from First Dose (Days)			
		14	21	28	35
1	1	1.5*	1	3.2	1.0
2	3	2.0*	2.5	3.6	3.6
3	5	3.0	4.7	3.75	4.0
4	Nil	Nil	Nil	Nil	Nil

* Only 2 animals per group. Other results are means of observations on 4 animals.

TABLE 2. Degree of Damage to Interlobular Bile Ductules

Group No.	Dose (ml)	Time of Reading from First Dose (Days)			
		14	21	28	35
1	1	2.0*	1.0	0.75	0.75
2	3	2.0*	2.7	2.2	1.0
3	5	3.5	3.2	1.5	1.5
4	Nil	Nil	Nil	Nil	Nil

* Only 2 animals per group. Other results are means of observations on 4 animals.

Biliary epithelial hyperplasia, damage to interlobular bile ductules, and vascular lesions were noted in all animals, with the exception of one guinea-pig in each of the 28- and 35-day divisions of group 1. Necrosis was observed in only 17 livers. The incidence of necrosis increased with higher dosage but was not a constant feature even at the highest dose level.

In any group the degree of development of lesions was roughly the same, although slight variations did occur.

Discussion

Under the conditions of this trial there was an obvious dose-response relation. Lower dosages consistently produced less response

regardless of the time of reading. On the other hand, the rate of development of lesions appeared to be affected by dose rate. It will be noted that maximal differences occurred at 21 days from the initiation of dosing and further that there was a close numerical relation between the degree of biliary epithelial hyperplasia and the dose administered.

It is possible that even better results could have been achieved had it been possible to administer the fungal material as a single dose. However, the material available did not lend itself to administration as a single dose. With a seven-day dosing schedule, for maximal differences and hence maximal sensitivity the optimal time of reading is 14 days after the cessation of dosing.

No great variability was observed between animals on any given dose rate examined at any particular time. This fact, together with the dose-response relation at 21 days from the initiation of feeding, suggests that this test procedure can be utilised for investigation of biological aspects of the hepato-toxic action of *P. chartarum*.

Acknowledgments

We should like to thank Drs R. H. Thornton and D. J. Ross of the Soil Bureau, Department of Scientific and Industrial Research, Taita, for supplies of *P. chartarum*; Messrs. F. K. Calverley and C. G. Fenwick, Misses E. L. Sheriden and E. O'Connell, and Mrs C. Van der Burg for dosing and looking after the experimental animals; and Mr A. L. Bryant for the photographs.

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MAGNESIUM DEFICIENCY IN PINE SEEDLINGS GROWING IN PUMICE SOIL NURSERIES

By G. M. Will, Forest Research Institute, New Zealand Forest Service, Rotorua

(*Received 28 March 1960*)

Summary

Magnesium deficiency, characterised by needle tip chlorosis, has occurred in seedlings of radiata pine, Corsican pine, and lodgepole pine in two New Zealand pumice soil nurseries. Exchangeable Mg in the soil was low, as were foliage contents. Magnesium sulphate gave a rapid response, but leached from the soil within a year. The response to dolomite was slower but longer lasting. Serpentine raised the level of Mg in the foliage to some extent, but did not increase exchangeable Mg in the soil to any appreciable extent. Foliar sprays of magnesium sulphate were very effective at some times of the year. The application of a low rate of ammonium sulphate increased the uptake of Mg as evidenced by increased foliage content and growth.

This is the first reported instance of Mg deficiency in: (1) forest trees in New Zealand; and (2) a virgin soil growing its first crop after cultivation.

Introduction

In Whakarewarewa and Kaingaroa forest nurseries in the Rotorua district needle tip chlorosis occurs in seedlings of radiata pine, Corsican pine, and lodgepole pine. Usually only the needle tips are yellow, but in severe cases the entire lengths of needles are affected and then the tips often show necrosis. These severely affected seedlings have a yellow and brown appearance and may lack green foliage completely. If these symptoms persist the seedlings die.

A variety of different causes of needle tip chlorosis in conifer seedlings has been reported from other countries. Bjorkman (1953) found that "yellow tip disease" in Norway spruce seedlings was associated with soils of high pH and an unfavourably low K/Ca ratio. Scots and Corsican pines show similar symptoms due to potassium deficiency (van Goor 1956). In most other reported cases, however, the yellowing of needle tips has been found to be due to a magnesium deficiency—either alone or in combination with other nutrient deficiencies. Becker-Dillenger (1937), Stone (1953), Lafond (1958), and Holstener-Jorgensen (1958) are amongst those who have reported yellow tipping due to magnesium deficiency alone. Nemec (1942) showed that yellow foliage from Scots pine and *Pinus strobus* contained low levels of both potassium and magnesium

but a high level of manganese. He suggested that the symptoms could have been caused by manganese toxicity. Voigt *et al.* (1958) found that an acute soil deficiency of both calcium and magnesium produced an intense yellowing of the outer portion of needles.

In New Zealand large quantities of lime and potassic and phosphatic fertilisers are used in agriculture, and some soils are deficient in minor elements. However, magnesium deficiency is uncommon and is usually induced by the use of large quantities of potassic fertilisers. It has been reported in apples in the Nelson district (Kidson *et al.* 1940); in tomato leaves in glass-houses (Johannesson 1951; McNaught and Gdanitz 1952); and in citrus fruits (Fletcher 1959); but not in forest trees or in virgin soils.

Symptoms

General

Needle tip chlorosis in pine seedlings has only been observed in the two forest nurseries in the Rotorua district: the Whakarewarewa nursery and the Kaingaroa nursery. The soil in both nurseries is derived from aerially deposited rhyolitic pumice.

Whakarewarewa nursery has been in use for more than 50 years. Continuous use and inadequate fertiliser applications have resulted in a loss of fertility. Amongst other signs of unthriftiness in seedlings, radiata pine (*Pinus radiata* D. Don) has shown slight to moderate yellowing of needles.

Kaingaroa nursery has been developed from virgin land within the last eight years. Here, where the soil type is Te Rere sand (Vucetich *et al.* 1961), chlorosis has been severe in radiata pine and Corsican pine (*P. nigra* Arnold) (larico) and has affected lodgepole pine (*P. contorta* Dougl.) as well. Chlorosis has sometimes been severe in areas growing their first crop of seedlings.

Detailed Descriptions

Radiata Pine. Symptoms closely parallel those for magnesium deficiency in seedlings in sand culture (Purnell 1958). In mild cases only the tips of needles near the top of the plant are affected. In more severe cases more needles and greater lengths of needles are affected. Also the yellow colour is more intense, giving a golden appearance to seedlings. Symptoms for both primary and secondary leaves (needles) are similar. This yellow tip on needles, with the remaining portion green, is in contrast with nitrogen, potassium, and iron deficiencies, when the chlorosis is more even over the whole lengths of needles (Purnell 1958; Will in press). Necrotic needle tips are a feature of the most severely affected plants. As symptoms become worse the growth rate decreases; in plants showing necrosis there is little or no growth.

Under some circumstances (discussed below under the heading "Occurrence") seedlings can partially overcome the deficiency. All new growth is then completely green, but the older needles remain yellow tipped. Where the deficiency is completely overcome all previ-

ously yellow foliage becomes green, leaving only the necrotic tips as evidence of the previous deficiency.

Corsican Pine. Symptoms are similar to those found in radiata pine except for the primary leaves. In these the yellow tip is separated from the green portion by a purple zone. In mild cases there may be only a purple tip to the foliage.

Lodgepole Pine. Symptoms are similar to those described for radiata pine but they have not been observed in primary leaves.

A slight yellowing and twisting of larch (*Larix decidua* Mill.) foliage may be due to the same cause, but no deficiency symptoms have been seen in the large numbers of Douglas fir (*Pseudotsuga taxifolia* (Poir) Britt.) growing in the same nurseries.

Occurrence

In 2/0* Corsican pine symptoms have intensified in the late summer and partially disappeared in the autumn. Voigt *et al.* (1958) reported that chlorosis appeared at a similar time of year in four species of pine and Norway spruce. On the other hand Nemec (1942) found that symptoms in Scots pine were worst in the spring and autumn. A similar cycle is apparent with 2/0* radiata pine, but other factors have an overriding influence. Frost, root wrenching, and drought all cause chlorosis to appear or intensify: on the other hand, periods of frequent rain will often cause a partial recovery.

Soil moisture plays a very important role in determining the severity of symptoms. In the dry summer of 1957-8 1/1** radiata pine in Whakarewarewa nursery showed severe chlorosis with stunting of growth. Similar seedlings in the same piece of ground in the 1958-59 summer, during which there were regular falls of rain, showed no chlorosis at all. Anything that tends to decrease the normal uptake of nutrients favours the appearance of chlorosis.

In all areas where chlorosis occurs it varies in intensity from bed to bed and within each bed. This results in uneven height growth and a patchwork effect—some parts being more yellow than others.

Experimental Procedure and Results

Foliar Sprays

In the summer of 1957-8 severe chlorosis in 1/0 and 1/1 radiata pine seedlings was accompanied by stunted growth in both Whakarewarewa and Kaingaroa nurseries. Three foliar applications of a 2% magnesium sulphate solution, at 10-day intervals, were sufficient to restore normal growth. Seedlings became almost completely green, while in untreated areas the chlorosis intensified. Foliar sprays were less successful at other times of year and with other species.

* 2/0 seedlings are in their second year in the seed beds.

** 1/1 seedlings have been lined out after one year in the seedbeds.

TABLE 1. EFFECTS OF FERTILISERS, 3 MONTHS AFTER APPLICATION, ON GROWTH OF CHLOROTIC 2/0 RADIATA PINE, KAINGAROA NURSERY

Fertiliser			Colour of Seedlings	Height of New Growth (in.)
Controls	None		More yellow foliage than green in controls	2
Non-magnesium treatment:—				
1. Effective	Ammonium sulphate	2 cwt/acre	Completely green	4
2. Non-effective	Superphosphate	3 cwt/ac	Colour similar to that of controls	2
	Sulphur	½ cwt/ac		
	Gypsum	1¼ cwt/ac		
	Lime	1 ton/ac		
	Potassium sulphate	2 cwt/ac	Worse than controls, some necrosis	< 2
Magnesium treatment:—				
1. Effective	Magnesium sulphate	2 cwt/ac	Completely green	5
	Mg + K sulphates	4 cwt/ac		
	N, P, K, Mg fertiliser *	7 cwt/ac		
2. Partially effective	Dolomite	1 ton/ac	Greener than controls; some lower needles yellow tipped	4
	Dolomite	1½ cwt/ac	Greener than controls; most lower needles yellow tipped	2–3
	Serpentine	¾ cwt/ac	Slightly greener than controls	2

* A 1 : 3 : 2 : 1 mixture of $(NH_4)_2SO_4$, superphosphate, K_2SO_4, and $MgSO_4$.

Fig. 1.—Chlorosis in 2/0 radiata pine seedlings, Kaingaroa Nursery. General view over part of 1958 fertiliser trials. Green plot in foreground treated 3 months previously with magnesium sulphate.

Fertiliser Trials

Fertiliser trials have so far been confined to radiata pine because this species shows the severest symptoms and makes up the larger part of plantings.

Kaingaroa Nursery. In the spring of 1958, beds of 2/0 seedlings were a golden yellow. A number of fertilisers were applied to a series of plots, and within a month responses in the form of greener foliage could be seen. After three months differences were very noticeable. (See Figs. 1 and 2.) These differences are summarised in Table 1. Magnesium sulphate alone and in combination with other fertilisers restored the colour and growth to normal. Both rates of dolomite had some effect, but ground serpentine, the other magnesium-containing treatment, had little or no effect. The detrimental effect of potassium sulphate appeared to be the same as that found by Holstener-Jorgensen (1958). The only non-magnesium fertiliser to have a beneficial effect was ammonium sulphate. This is contrary to the findings of Mulder (1955) that magnesium uptake is hindered by ammonium salts but aided by nitrates. Other trials designed to test the effects of various nitrogen fertilisers on chlorosis were commenced. In some cases low rates of application of ammonium sulphate (2 cwt/acre or less) caused the chlorosis to disappear, but higher rates intensified the quantity of chlorosis present. This increased chlorosis was corrected by an application of magnesium sulphate. In view of Mulder's findings, it is suggested that, under suitable conditions, low rates of ammonium N may be quickly nitrified, and the beneficial effects that follow are a nitrate response.

TABLE 2. Height Increments and Foliage Mineral Compositions (% Dry Matter) of 2/0 Radiata Pine Seedlings, Kaingaroa Nursery, 9 Months after Application of Fertilisers

	Mean Height* Increment (in.)	Ca		Mg		K		P	
		New Foliage	1-Yr-old Foliage	New Foliage	1-Yr-old Foliage	New Foliage	1-Yr-old Foliage	New Foliage	1-Yr-old Foliage
Controls	7.41	.18	.17	.09	.06	1.14	.85	.23	.18
$MgSO_4$	12.56	.11	.18	.14	.09	1.13	.99	.24	.15
$MgSO_4 + K_2SO_4$	13.00	.10	.17	.16	.09	1.30	1.44	.27	.17
N. P. K. Mg fertiliser **	13.19	.17	.19	.11	.08	1.32	1.34	.25	.16
Dolomite (1 ton)	12.28	.20	.27	.12	.07	1.23	1.00	.30	.16
Dolomite (1½ cwt)	11.33	—	—	—	—	—	—	—	—
Serpentine	8.00	.19	.23	.11	.07	1.07	1.00	.19	.17
$(NH_4)_2SO_4$	11.48	.08	.22	.14	.09	1.08	1.00	.28	.17

* The 95% probable limits of error for the controls and treatments are ± 0.75 and ± 0.65 respectively. Students' t-test gave differences between controls and treatments greater than 8 in all cases except serpentine. The other treatments are therefore highly significantly better than the controls.

** See Table 1.

The chemical analyses were carried out by the methods of Metson (1956), using a versenate titration for calcuim and magnesium.

Towards the end of the growing season all seedlings had green tops, but only those in the magnesium fertiliser and ammonium sulphate plots were completely green. These had maintained a higher growth rate. (See Table 2.) Also the serpentine plot, at this stage, was noticeably greener than the controls. Table 2 gives

Fig. 2.—Response to magnesium sulphate by chlorotic radiata pine seedlings, Kaingaroa Nursery. Untreated controls above and below magnesium sulphate plot.

the results of foliage analysis on: (1) foliage present when treatments were applied; and (2) subsequent growth. Nitrogen values are not included, as they were similar for all treatments: 1.9% in young foliage, 1.4% in older foliage.

Calcium and potassium levels were higher in the foliage where fertilisers containing these elements had been applied. But the most marked effects were: (1) the increase in magnesium content in plots treated with magnesium; and (2) the relation of this increased magnesium content to growth rate. Although the application of serpentine gave no significant increase in height growth, the improvement in colour and the rise in foliar magnesium should be noted.

TABLE 3. pH and Exchangeable Cations (me.%) in Kaingaroa Nursery Soil 9 Months after the Application of Fertilisers

Fertiliser	pH	Exchangeable Cations (me.%)		
		Ca	Mg	K
Controls (untreated soil)	5.0	.25	.10	.16
$MgSO_4$	5.0	.20	.10	.16
$MgSO_4 + K_2SO_4$	5.0	.20	.10	.26
N, P, K, Mg fertiliser	5.4	1.40	.35	.25
Dolomite 1 ton	6.5	5.50	1.50	.18
Dolomite 1½ cwt	5.6	.95	.55	.17
Serpentine	5.0	.20	.10	.18
$(NH_4)_2SO_4$	5.1	.25	.10	.16

The analyses were carried out by the methods of Metson (1956), using a versenate titration for calcium and magnesium.

In Table 3 are given the levels of exchangeable cations present in the top 4 in. of soil at the time foliage samples were taken, i.e., about nine months after treatment. The level of exchangeable magnesium in the untreated soil (0.10 me.%) is low when compared with the critical level of about 0.20 me.% found for apples in the Nelson district (Kidson *et al.* 1940). Except where superphosphate was applied, and the pH, calcium, and magnesium values were all higher, no additional exchangeable magnesium was left in the soil nine months after the application of a dressing of 2 cwt/ac of magnesium sulphate (only 1 cwt in the super plot). On the other hand, where an equivalent amount of magnesium was applied as dolomite (1½ cwt), the amount of leaching was negligible. As would be expected, even higher values resulted from the application of 1 ton of dolomite, and these may subsequently increase still further as there was still free carbonate in the soil. The seedlings growing in the serpentine plot had higher foliage levels of magnesium, but

the exchangeable magnesium in the soil remained the same as in the controls.

Although all the magnesium not taken up from a dressing of magnesium sulphate by seedlings was leached from the soil in less than nine months, the figures in Table 3 show that potassium applied as potassium sulphate remained in the soil for a longer period.

Whakarewarewa Nursery. In the spring of 1958 a replicated trial of soil sterilisation and organic and inorganic fertilisers was laid down. Radiata pine was included among the species sown. Although seedlings in all the plots treated with magnesium fertilisers were a noticeably darker green than the remainder, only in one case was there a significant increase in height. Treatment of this soil with chloropicrin results in greatly improved growth (Thulin *et al.* 1958). The use of dolomite at the rate of 2 cwt/ac gave a further increase in height growth, significant at the 1% level. Overall, magnesium treatments gave an increase in height growth over their non-magnesium counterparts significant at the 10% level.

Conclusions

Low levels of exchangeable magnesium in the soil have caused chlorosis in pine seedlings in Whakarewarewa and Kaingaroa nurseries. The low exchangeable magnesium level in Whakarewarewa nursery has resulted from many years of intensive cultivation, but the virgin soil in Kaingaroa nursery was just as deficient.

A level of 0.10 me.% exchangeable magnesium in the soil is insufficient to maintain healthy growth: the critical level is probably nearer the 0.20 me.% found for apple trees growing on another soil type.

In the current year's foliage of radiata pine seedlings 0.12%–0.14% magnesium indicates an adequate supply.

Deficiency symptoms are intensified when the normal uptake of nutrients is decreased by drought, root wrenching, or frost.

The application of 2 cwt/ac of magnesium sulphate is sufficient to restore colour and growth to normal within a few weeks, but it leaches rapidly from the soil. Because of this the slower acting dolomite is a more efficient means of raising the level of exchangeable magnesium. Ground serpentine applied at an equivalent rate improves seedling growth a little but does not increase the exchangeable magnesium in the soil in the first year after application.

Low rates of application of ammonium sulphate can have a beneficial effect on the uptake of magnesium. The reason is not fully understood, but it is suggested that the cause is a nitrate response following nitrification.

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A SURVEY OF SOME PHYSICAL PROPERTIES OF NEW ZEALAND SOILS FROM GREYWACKE PARENT MATERIAL

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Summary

Measurements of physical characteristics likely to influence plant growth are presented for 12 soil types derived from greywacke rock. Four modal sites were examined for each type, two under pasture and two in the virgin state. In general, results were non-separable as between one site and four sites, but confidence limits were such that the measurements could be used to assess physical suitability for growth. (Work on the relation of measurements described here to plant growth is briefly reviewed and tentative gradings suggested.) Aspects of field capacity estimation and percolation rate measurement are briefly discussed.

Introduction

Certain physical properties of soil are important because of their bearing on such subjects as plant growth, irrigation, and soil puddling. However, little previous systematic or quantitative work on these properties has been done in New Zealand. Some work on pumice soils (Packard 1957) and "pakihi" soils (McDonald 1955) has been published, but for much of the country no quantitative physical information is available. The project reported here is an attempt to characterise broadly the physical properties of some New Zealand soils and, at the same time, to determine the physical differences, if any, which exist between soils from under virgin cover and under pasture.

Selection of Soils and Sites

The soils of New Zealand have been classified by Taylor (1948) into approximately 60 *genetic soil types*. From each genetic type a representative *soil type* (hereinafter called "type") was selected. This paper deals, in particular, with soils of greywacke parent material. Four sites, two under the best available pasture and two in the virgin condition (or as little disturbed as possible) were chosen for each type. The sites were considered to be modal rather than to represent the range of soils grouped under type name. General locations of sites and descriptions of types are given in Table 1. In all tables the types are

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TABLE 1. SOIL TYPE, DESCRIPTION, AND LOCATION

Soil Type	Description	
	A Horizon	B Horizon
Haldon steepland soil	6 in. dark brown gravelly silt loam, firm to friable, moderately developed medium and fine granular and nutty structures, many roots.	10 in. yellowish brown silty clay loam, firm, weakly developed medium to coarse nutty structure.
Kaikoura steepland soil	5 in. dark brown silt loam, very friable, strongly developed fine nutty and crumb structure, many roots.	12 in. yellowish brown stony silt loam, friable, moderately developed fine nutty structure, many roots.
Ruahine steepland soil	6 in. greyish brown stony silt loam, friable, moderately developed fine nutty structure (under forest), weakly developed fine nutty structure with some cast granular (under pasture).	12 in. yellowish brown, stony silty clay loam, friable, moderately developed fine blocky structure.
Makara steepland soil	7 in. dark greyish brown stony silt loam, friable, strongly developed medium nutty structure (under forest), moderately developed medium nutty structure with some cast granular (under pasture).	14 in. dark yellowish brown stony silt loam, friable, moderately developed medium nutty structure.
Te Ranga* steepland soil	¼–1 in. dark greyish brown stony loam, very friable, on 6 in. brownish yellow stony clay loam, friable, moderately developed fine and medium nutty structure, many roots.	7 in. yellow stony clay, friable, moderately developed medium and fine nutty structure.
Owaka silt loam	8 in. brown silt loam, moderately developed fine to medium nutty structure.	15 in. yellowish brown silt loam, blocky structure.
Belmont silt loam	10 in. dark brown silt loam, friable, strongly developed nutty structure, abundant roots.	8 in. dark yellowish brown to yellowish brown silty clay loam, firm, moderately developed fine nutty to blocky structure, few roots.
Korokoro silt loam	9 in. dark brown to brown friable silt loam, moderately developed fine to medium granular to nutty structure, abundant roots.	12 in. brownish yellow firm clay loam with strongly developed medium nutty structure, nutty aggregates have very thin brown coating, many brown worm casts and roots.
Taita clay loam	5–6 in. greyish brown clay loam, firm, moderately developed fine to medium nutty structure, many roots, few faint and fine reddish yellow mottles.	8–10 in. brownish yellow clay loam, moderately compact, moderate medium to coarse nutty structure, many distinct reddish yellow mottles, few roots and stones.
Renata silt loam	(a) Under forest: 4 in. reddish grey silt loam, friable, moderately developed medium nutty structure.	10 in brown to yellowish brown silt loam, firm, moderately developed fine blocky structure.
	(b) Under pasture: 4 in. dark reddish brown silt loam, friable, moderately developed very fine granular and medium nutty structure.	10 in. dark reddish brown and dark yellowish brown mottled silt loam, moderately developed medium nutty structure.
Marua clay loam	3–4 in. dark greyish brown clay loam, friable, compound structures of moderately to weakly developed fine nutty and fine cast granular, abundant grass roots.	7 in. pale yellowish brown clay, moderately compact, moderately developed medium and fine nutty structure, many grass roots.
Rangiora clay	4 in. dark grey silty clay loam, firm, weakly developed fine nutty structure with fine cast granular, many roots.	16 in. brownish yellow heavy clay, very firm, moderately developed coarse nutty structure combined into prisms.

* The A horizon samples for Te Ranga steepland soil included the ¼ in.–1 in. layer.

Underlying Material	Genetic Description				Location	Slope
	Genetic No.	Soil	Degree of Weathering	Degree of Leaching		
Greywacke rubble	21b	Skeletal yellow grey earth	Moderate	Weak	Waipara County	20–30°
Greywacke rubble and rock	21c	Skeletal yellow brn. earth	Weak	Strong	Tawera County	33°
Weathering greywacke	21D	Ditto	Moderate	Strong	Hutt County	35°
Weathering greywacke rock	21d	Ditto	Moderate	Moderate	Makara County	35°
Yellow stony clay from greywacke	21e	Ditto	Strong	Moderate	Whangarei County	30–38°
Heavy silt loam from greywacke	5B	Yellow brown earth	Submoderate	Moderate to strong	Clutha County	6– 8°
Yellowish brown fine sandy loam, firm and massive from greywacke	5B	Ditto	Submoderate	Moderate to strong	Hutt County	10–14°
Yellowish brown stony clay loam from weathering greywacke	5b	Ditto	Moderate	Moderate	Makara County	8–12°
Yellow brown stony clay loam from greywacke	5c	Ditto	Moderate	Strong	Lower Hutt City	8–14°
On weathering greywacke and solifluction silts On solifluction silts from greywacke	7a	Podzolised yellow brown earth	Weak to moderate	Strong	Hutt County	6–12°
Brownish yellow clay from greywacke	6b	Yellow brown earth	Strong	Moderate	Whangarei County	8–14°
Very pale brown clay from weathering greywacke	6c	Ditto	Strong	Strong	Whangarei County	6–12°

set out in the following order:

(1) The steepland group of soils, concluding with Te Ranga steepland soil.

(2) The group on rolling slopes, concluding with Rangiora clay.

The soils within each group are set out in order of weathering.

Sampling

In view of the amount of work necessary, it was decided to sample the A and B horizons only. This, however, does, in general, cover the rooting range of grasses. Descriptions of underlying materials are presented as part of Table 1.

In the light of some preliminary work it appeared that differences which would be statistically significant and of agricultural importance should probably be detected by the following sampling programme. From each horizon at a site, four undisturbed samples were taken in brass sampling cans 4 in. in diameter and 3 in. high. Also, two samples of soil were collected in plastic bags, each sample being a composite from two positions at a site.

For some types one undisturbed B horizon core (6 in. long by 4 in. diameter) was taken for percolation rate measurement. The area of a site varied but would not exceed 20 yd square. All sites were on either hilly or rolling country and were chosen to avoid possible swampy or dry areas, i.e., generally they were at least 20–30 yd below adjacent hill-tops and well above the valley bottoms. Care was taken to sample on convex, rather than concave, slopes. Samples were taken only if they appeared to conform to the pedologist's description of the modal profile for the type.

Cyclic variation in physical properties, particularly aggregate stability, has often been reported, for example, by Clement and Williams (1958). These authors suggest that the deleterious effect of this variation on soil comparisons may be reduced by collecting all samples at the one season of the year. This procedure was not practicable in this survey. However, Heinonen and Pukkala (1954) reported strong positive correlations between field moisture content and the measurements for aggregate stability and total porosity, and recommended that samples for comparision purposes should be collected under conditions of uniform moisture, e.g., field capacity. In most instances when samples were collected during the present survey the soils were damp but not saturated. The writer would judge that in many cases the sites were approximately at field capacity and it is considered that errors arising from cyclic variation do not seriously affect the general conclusions drawn from the present work.

Experimental

The total porosity of the constant-volume undisturbed samples was obtained by calculation from: (1) the specific gravity of the sample—measured by pycnometer bottle using toluene as the displacing liquid; and (2) the oven-dry weight at 105°c.

Macro-porosity was determined by draining the undisturbed samples at a tension of 50 cm of water for 24 hours.

Field capacity was obtained, either by measurement of the soil moisture when weather conditions were deemed suitable, or by calculation from a laboratory measurement using a regression equation, as discussed later.

Percolation rate measurements were carried out on the 6 in. × 4 in. undisturbed core, using a head of ½ in.–1 in. of water.

Soil from the plastic bag samples in the field-moist condition was passed through a No. 4 A.S.T.M. sieve (aperture 4.76 mm) and this material was used in the following measurements.

Two pF/moisture content values were obtained, that for pF 2.31 by draining the sieved soil under the appropriate tension on a silt membrane by a procedure similar to that of Scholfield (1935), that for pF 4.19 (the wilting point) by the pressure membrane method (Richards 1947).*

Mechanical analysis was carried out by the hydrometer method and aggregate stability by a modification of the wet-sieving method.

The following points should be noted with regard to the measurements made on the sieved materials:

Most of the soils were non-stony, i.e., they contained no fundamental particles greater than 2 mm diameter. In the case of stony soils the weight of fundamental particles, greater than 2 mm diameter which passed the 4.76 mm mesh and were thus included in a particular measurement, was determined. Corrections were then made so that results could be stated on the uniform bases as presented.

The assumption that the stones do not contain moisture can introduce errors in the results for the pF/moisture content values, particularly where large quantities of porous stones are present. However, in the present work: (1) the maximum moisture content for any stones was 10.6% by weight; (2) the available moisture content figures (being a difference) can be regarded as correct in that the stone moisture content is unlikely to change between the two relevant tension states; and (3) large variability exists in the quantity of stones in samples from any given site. Therefore, following Saidelman (1957), calculations have been based on the assumption that stones do not contain moisture.

Results and Discussion

Results are presented in Tables 2 and 3 and are discussed for individual types only insofar as they have a bearing on the general conclusions. However, a tentative assessment (presented later) of the relation of the physical measurements to plant growth will permit evaluation of soil type from this aspect.

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* Available moisture is defined as the difference between wilting point and field capacity, whether measured or estimated.

TABLE 2. Means and Standard Errors of Physical Measurements
(Figures in brackets are numbers of individual measurements)

Type	Horizon	Site	Mechanical Analysis Range And Mean %				Stones (> 2 mm) % By Vol.
			2–0.2 mm	0.2–0.02 mm	0.02-0.002 mm	< 0.002 mm	
Haldon steepland soil	A	1, 2, 4 3	10–30, 18	25–38, 31	24–28, 26	19–33, 25	3.8±0.7 (16)
	B	1, 2, 4 3	14–31, 20	24–37, 30	19–27, 24	20–39, 25	8.0±1.2 (16)
Kaikoura steepland soil	A	1, 3, 4 2	11–15, 13	38–44, 40	26–27, 26	18–22, 20	3.8±1.3 (12)
			44	27	18	11	7.9±3.4 (4)
	B	1, 3, 4 2	11–21, 15	32–36, 34	28–30, 29	19–24, 21	10.6±1.5 (12)
			42	29	18	11	13.0±3.6 (4)
Ruahine steepland soil	A	2, 3, 4 1	5–15, 11	30–41, 35	27–32, 30	20–28, 24	5.0±1.3*** (8)
			21	33	25	21	11.8±3.5 (4)
	B	2, 3, 4 1	7–15, 11	29–38, 34	24–31, 29	24–27, 26	4.5±0.7*** (8)
			18	32	24	26	20.8±8.1 (4)
Makara steepland soil	A	1, 2, 4 3	2–13, 8	24–47, 36	27–36, 31	24–27, 26	8.5±1.8 (12)
			35	26	21	18	28.9±5.0 (4)
	B	1, 2, 4 3	2–8, 6	37–46, 42	26–30, 28	20–26, 24	12.7±2.5† (8)
			24	29	26	21	18.8±4.2 (4)
Te Ranga steepland soil	A	1, 3, 4††	3–11, 7	10–15, 13	23–28, 26	51–59, 54	
	B	1, 2, 3, 4	2–10, 5	8–16, 13	23–31, 26	49–64, 56	
Owaka silt loam	A	1, 2, 3 4	2–8, 5	35–42, 39	25–31, 28	25–31, 27	
			2	40	40	18	
	B	1, 2, 3 4	2–9, 6	35–42, 39	24–30, 28	25–28, 27	
			2	41	37	20	
Belmont silt loam	A	1, 2, 4 3	1–6, 3	30–39, 35	32–38, 35	22–32, 26	
	B	1, 2, 3, 4	0–5, 3	32–63, 47	29–34, 32	8–29, 18	
Korokoro silt loam	A	1, 2, 3, 4	1–16, 6	26–37, 30	25–36, 30	28–39, 33	
	B	1, 2, 3, 4	1–20, 7	25–39, 28	25–39, 31	20–43, 32	
Taita clay loam	A	1, 2, 3, 4	4-6, 5	36–38, 37	25–35, 31	24–31, 27	
	B	1, 2, 3, 4	4-6, 5	32–34, 33	22–29, 27	33–38, 35	
Renata silt loam	A	1, 2, 3 4	1–3, 2	44–46, 45	32–36, 33	19-21, 20	
	B	1, 2, 3, 4	1–7, 3	36–48, 43	32–35, 33	19-22, 20	
Marua clay loam	A	1, 2, 3, 4	1–4, 2	14–24, 20	27–32, 29	41–57, 49	
	B	1, 2, 3, 4	1–4, 2	10–23, 15	24–29, 27	49–62, 55	
Rangiora clay	A	2, 3, 4 1	1–4, 2	9–26, 16	22–38, 30	38-66, 52	
	B	1, 2, 3, 4	1–3, 2	8–21, 12	20–36, 28	47–71, 58	

* Sites 1 and 2. ** Sites 3 and 4. *** No stones at Site 2. † Stones at Site 2, 0.7 ± 0.4. †† Negligible A horizon at Site 2.

Density of Stones g/cm³	Degree of Aggregation %	Available Moisture % By Vol.	Bulk Density g/cm³	Total Porosity %	Macroporosity %	Percolation Rate cm/hr
2.71	63.3±2.5 (12) 43.9±0.6 (4)	14.9±0.8 (4)	1.14±0.03 (12) 1.31±0.02 (4)	55.4±0.8 (12) 48.7±0.5 (4)	20.0±1.1*(8) 11.4±0.5**(8)	
	40.3±2.7 (12) 9.4±2.4 (4)	8.3±1.7 (4)	1.42±0.02 (16)	46.1±0.8 (16)	12.4±1.1 (16)	
2.72	61.0±2.4 (12) 36.5±2.3 (4)	20.2±2.0 (3) 11.7 (1)	0.62±0.03 (12) 0.78±0.08 (4)	74.3±1.0 (12) 69.0±2.8 (4)	26.5±1.6 (12) 30.1±0.9 (4)	
	46.7±2.9 (12) 28.0±3.4 (4)	16.7±2.0 (3) 12.9 (1)	0.87±0.03 (12) 0.97±0.07 (4)	65.9±1.2 (12) 62.4±2.5 (4)	22.1±1.8 (12) 18.1±3.7 (4)	
2.63	73.7±1.4 (12) 64.5±5.2 (4)	16.4±3.3 (3) 16.4 (1)	0.67±0.02 (12) 1.19±0.02 (4)	71.3±1.2 (12) 52.5±0.9 (4)	23.8±1.9 (12) 16.4±3.5 (4)	
	58.2±2.2 (12) 41.2±5.6 (4)	13.1±2.4 (3) 9.8 (1)	0.77±0.04 (12) 1.46±0.05 (4)	69.1±1.4 (12) 43.7±1.9 (4)	22.1±3.9 (12) 10.6±1.4 (4)	15.7
2.69	52.8±3.8 (12) 40.9±1.3 (4)	19.6±1.5 (3) 12.9 (1)	1.14±0.03 (12) 1.36±0.04 (4)	55.1±1.2 (12) 48.2±1.1 (4)	14.1±1.4 (12) 15.7±1.1 (4)	
	31.5±2.4 (12) 16.1±1.8 (4)	16.3±3.3 (3) 13.3 (1)	1.27±0.3 (12) 1.48±0.04 (4)	50.8±1.2 (12) 44.3±1.5 (4)	13.7±0.9 (12) 13.5±0.8 (4)	14.8
	78.6±1.9 (12)	18.8±1.2 (3)	0.93±0.02 (12)	61.8±1.0 (12)	14.1±1.0 (12)	
	62.1±1.1 (16)	10.6±0.2 (4)	1.06±0.02 (16)	58.5±0.9 (16)	12.1±0.9 (16)	
	56.2±0.9 (12) 47.6±2.3 (4)	16.1±0.6 (3) 17.5 (1)	1.04±0.03 (12) 0.77±0.01 (4)	58.6±1.0 (12) 67.6±0.5 (4)	8.6±0.8 (12) 7.3±0.7 (4)	
	40.7±1.7 (12) 28.4±4.0 (4)	9.8±0.8 (3) 14.0 (1)	1.32±0.02 (12) 1.10±0.05 (4)	49.8±0.7 (12) 57.4±1.8 (4)	5.8±0.3 (12) 7.3±0.8 (4)	
	62.5±1.5 (16)	24.6±1.5 (4)	0.97±0.02 (16)	59.6±0.7 (16)	8.2±0.8 (12) 4.2±0.2 (4)	
	43.7±1.2 (16)	14.3±2.8 (4)	1.08±0.03 (16)	57.3±1.1 (16)	12.5±0.9 (16)	2.79
	54.5±1.8 (16)	20.2±1.7 (4)	1.04±0.02 (16)	57.3±0.8 (16)	10.4±1.1 (16)	
	33.7±3.4 (16)	15.8±1.2 (4)	1.34±0.02 (16)	48.3±0.6 (16)	5.7±0.4 (16)	0.21
	41.1±2.5 (16)	23.0±1.8 (4)	1.07±0.02 (16)	55.9±0.7 (16)	11.8±0.8 (16)	
	24.2±1.2 (16)	8.3±1.4 (4)	1.39±0.01 (16)	45.8±0.5 (16)	4.8±0.6 (16)	0.09
	67.9±2.2 (12) 39.5±1.5 (4)	21.1±2.0 (4)	0.79±0.03 (16)	66.0±1.0 (16)	11.0±1.1 (16)	
	47.5±3.7 (16)	20.4±1.0 (4)	0.91±0.05 (16)	63.8±1.7 (16)	8.7±0.7 (16)	0.18
	70.9±2.6 (16)	25.2±1.4 (4)	0.90±0.02 (16)	62.6±1.0 (16)	12.5±1.2 (16)	
	55.5±1.2 (16)	9.4±1.3 (3)	1.11±0.02 (16)	56.4±0.8 (16)	7.6±0.6 (16)	
	64.0±2.3 (16)	16.3±1.0 (4)	0.93±0.02 (12) 1.13±0.02 (4)	61.4±0.8 (12) 53.7±0.5 (4)	7.9±0.6 (16)	
	53.9±1.6 (16)	8.3±1.9 (4)	1.15±0.02 (16)	54.7±0.7 (16)	5.3±0.5 (16)	

Mechanical analyses results based on oven-dried (105°c), H_2O_2-treated material.

Degree of aggregation $= \frac{\text{aggregates} > 0.25 \text{ mm composed of particles} < 0.25 \text{ mm}}{\text{material} < 2.00 \text{ mm.}}$

Separation of Types, and Differences Between Pasture and Virgin Soils

As mentioned, four sites were sampled for each soil type. A statistical test (Grubbs and Weaver 1947) showed that, for some types, measurements for a particular site were significantly different from those for the other three sites, and results for these measurements are listed separately in Table 2. The criteria used for regarding a site as discrepant compared with the rest of the type were:

(1) Several measurements for the site must be significantly different as compared with the corresponding horizons in the other sites of the type.

(2) Significant differences as above must be present in both horizons. (For example, site 3, Haldon steepland soil, is not regarded as discrepant, since results from the B horizon are, in general, consistent with those from the other sites.)

Regarding the discrepant sites, Kaikoura steepland soil, site 2 was of noticeably coarser texture and situated at the bottom of a scree, but it was selected because of other experimental work in progress; for Ruahine steepland soil, site 1, and Makara steepland soil, site 3, the areas were selected, but the sites were not actually examined by pedologists; for only one site—Owaka silt loam, site 4—are the physical measurements and the pedologist's judgment of a modal site at variance, and in this instance the pedologist was unfamiliar with the district.

In no type are the two virgin sites discrepant—by the above criteria—as compared with the two pasture sites. However, it should be pointed out that results from forest sites may be biased, since it was necessary to avoid roots which in some cases appeared to occupy a considerable volume.

In general, it would appear that a selection by a pedologist of a group of four modal sites—whether virgin or pasture, or a combination of both—for greywacke soil types may be characterised physically within the limits implicit in Table 2. As will be seen in the next section, these limits are such that the characterisations are likely to be significant as regards plant growth.

Relation Between Physical Measurements and Plant Growth

The soil physical factors which are of predominant importance in the growth of plants are air and moisture relations, root penetrability, and temperature. The measurements used in this survey should be regarded as attempts to gain significant information about the first three of these factors. In overseas work, similar measurements have been related to yield, root penetration, and seedling emergence of such plants as sunflowers, sugar beet, wheat, and potatoes. But little information is available regarding the relations of such measurements to pasture growth, although

the factors they reflect are no doubt relevant. A tentative evaluation of the measurements (as below) requires the arbitrary assumption that they also relate to pasture in a similar manner. The data available, covering as they do a range of plants, soil textures, and soil parent materials, indicate that the groupings must be kept fairly broad in order to rate the measurements on this merit basis. The comments below may be relevant to soils from a range of parent materials and are not intended to apply especially to soils derived from greywacke.

TABLE 3. Field Capacities and Wilting Points

Type	Site	Range and Mean % By Vol.			
		Field Capacity		Wilting Point	
		Horizon		Horizon	
		A	B	A	B
Haldon steepland soil	1, 2, 3, 4	30.6–36.4, 34.4	20.3–31.2, 26.3	16.1–22.7, 19.5	15.8–20.3, 18.0
Kaikoura steepland soil	1, 3, 4 2	31.9–40.4, 34.9 21.1	26.8–36.2, 31.5 26.7	12.7–17.2, 14.7 9.4	12.7–16.4, 14.8 13.8
Ruahine steepland soil	2, 3, 4 1	31.6–47.8, 40.0 30.7	31.2–43.7, 38.3 26.3	19.3–25.9, 23.6 14.3	21.4–27.6, 25.2 16.5
Makara steepland soil	1, 2, 4 3	33.2–38.3, 36.0 20.4	25.3–39.8, 32.8 26.2	14.8–18.8, 16.4 7.5	13.7–18.4, 16.5 12.9
Te Ranga steepland soil	1, 2, 3, 4	45.1–51.3, 48.5	41.3–44.9, 43.4	27.7–33.0, 29.7	31.0–34.7, 32.8
Owaka silt loam	1, 2, 3 4	48.9–53.7*, 50.5 54.9*	41.7–48.5*, 44.6 49.9*	32.9–37.1, 34.4 37.4	30.8–38.7, 34.8 35.9
Belmont silt loam	1, 2, 3, 4	43.1–54.1, 49.3	34.1–47.5, 40.4	22.6–29.1, 24.7	21.2–29.7, 26.1
Korokoro silt loam	1, 2, 3, 4	37.3–44.6, 41.6	33.7–46.5, 40.4	20.7–22.9, 21.4	19.0–29.0, 24.6
Taita clay loam	1, 2, 3, 4	41.0–47.8, 44.4	32.9–38.0, 35.7	19.5–22.9, 21.4	21.8–29.5, 27.4
Renata silt loam	1, 2, 3, 4	39.6–50.0, 44.8	44.6–54.2, 50.6	22.5–24.7, 23.7	25.3–35.5, 30.2
Marua clay loam	1, 2, 3, 4	52.0–57.7, 54.4	41.9–44.2, 42.7	25.2–32.1, 29.2	29.9–36.1, 33.3
Rangiora clay	1, 2, 3, 4	41.7–52.0, 46.9	38.1–47.6, 43.7	24.0–37.8, 30.6	32.1–40.1, 35.4

* Estimated.

Texture

Work by Dunn and Lyford (1946) indicated that the effect of texture, *per se,* on plant growth was minor, although plants in very coarse material (gravel—average 5 mm diam.) gave reduced yields. It does not seem necessary to assess this factor for growth.

Aggregation

Martin *et al.* (1955) state "there is little evidence that aggregates have any direct influence on plant growth except as they modify the pore spaces in the soil." For this reason measurements usually are concerned not with the shapes of the aggregates but rather with soil porosity (see later) and the stability of the aggregates.

Since aggregate stability is only one of the factors affecting pore characteristics, it is not feasible to attempt to define valid growth rating groups for this measurement. Also the results of aggregate stability methods are susceptible to technique; there is as yet no standard method. Thus it is unwise to compare results from different investigators. However, although Hubbell and Staten (1951) found no significant correlation between aggregate stability and yield, generally investigation has shown that an increase in the percentage of stable aggregates has been associated with increased growth (Martin 1953; Page and Willard 1946; van Bavel and Schaller 1950; Bushnell 1953).

Total Porosity

The results of Bushnell (1953) and Pendleton (1950), who considered root growth in potatoes and sugar beet, respectively, indicate the possibility of the following ratings for growth for total porosity:—

	%
Good	> 60.
Fair	50–60.
Poor	< 50.

Bulk Density

Bulk density can be regarded as a function of total porosity and particle density but is often considered in its own right. Those who have considered bulk density in relation to growth include Pendleton (1950), McIntyre (1955), Hanks and Thorp (1956), Smith and Cook (1947), and Veihmeyer and Hendrickson (1948). Generally, the soil particle densities, where reported by these workers, are within the same range as those of the present project. Results vary, but it would appear that bulk densities of

> 1.3 in medium and fine-textured soils, and

> 1.6 in sandy soils,

are likely to be detrimental to growth. Generally, low bulk densities are desirable, since usually they indicate the higher levels of total porosity.

Macroporosity

The larger-sized pores (macropores) in soil have been investigated by draining undisturbed samples at tensions between 40 and 100 cm of water or by measuring the water-free air space in the soil at appropriate times. Relations between growth and macroporosity have been reported by Page and Willard (1946), Baver and Farnsworth (1940), Bushnell (1953), Miller and Mazurak (1958), Pendleton (1950), McIntyre (1955), Hanks and Thorp (1956), and Dunn and Lyford (1946). Most of these deal with non-pasture plants in field or pot experiments, but Kopecky (1928) cites desirable macroporosities for grass.

Since macroporosity figures tend to indicate aeration and water movement properties of the soil, high figures are desirable. Often high macroporosities are associated with high total porosities, in which case moisture storage figures are likely to be satisfactory. However, a high macroporosity associated with low total porosity may indicate a soil with poor storage.

The following are possible ratings suggested by consideration of the work mentioned above:—

	%
Good	> 12.
Fair	6–12.
Poor	< 6.

Moisture

Stanhill (1957), in a recent review of the literature relating soil moisture and plant growth, found "that, in over 80% of the experiments, growth was affected by differences in the amount of available water depleted before the soil was re-wetted." However, only two of the experiments under review dealt with grass, and in only one did the result support the above statement.

Low and Armitage (1959) found that maximum dry matter production from a pasture was possible at pFs from 2 to 3.34 and that, even at pF 3.80, the reduction in production was small.

It is, of course, not necessary for growth that the moisture storage should be high, provided that a supply of moisture is available. However, the higher the available moisture storage, the longer the likely period of growth after rain ceases. The following ratings for available moisture for topsoil are suggested:—

	% by vol.
High	> 22.
Medium	14–22.
Low	< 14.

The figures suggested above for macroporosity and available moisture are similar to, although slightly higher than, those presented by Stirk (1957) for similar rating categories.

Homogeneity of Sites in a Type

In the light of the previous discussion, it would appear that the confidence limits for the results in Table 2 are such that the results may probably be regarded as satisfactorily characterising some of the physical properties likely to affect growth.

A statistical test (Tippett 1925) was applied to each type to compare the variation of measurement results over the whole type with the variation of results for each site in the type. Except as stated below, no significant difference was detected between the variations.

In 13 horizons results for *measured* field capacity also agreed with this statement; there were four exceptions.

The test was not applied to the pF/moisture content results, since the number of samples per site was not sufficient for reliable testing.

The results of this test, together with the failure of the site means to prove separable from the type means, as discussed earlier, suggest that, in general, for soils from greywacke it should be possible to characterise physically—as herein—the modal profile for a type by investigation of the soils from one site only.

Within broad limits field differentiation of soil types is based on morphological features. It would appear unlikely that two soil sites having very dissimilar features would be included in one type, still more so that they would both be regarded as being modal sites for the type. Therefore, since the physical measurements reflect such features, it would appear unlikely that the measurements would distinguish between modal sites for the same types—including those from parent material other than greywacke. From this viewpoint it does not seem surprising that no difference was detected between modal sites for virgin and for pasture. There is the possibility that the development of a site once regarded as modal may be accompanied by changes in field features* and that this site may be selected for comparison with modal sites on undeveloped areas. In such a case it is possible that a difference between virgin and developed sites may be detected by physical measurements.

Physical Results in Relation to Soil Classification

In the following discussion results for sites which are regarded as discrepant are not considered.

Characteristics of Haldon steepland soil—the only yellow-grey earth included in this investigation—are the high bulk density and low total porosity of the subsoil. Available moisture is low for both topsoil and subsoil.

In order of increasing weathering the other steepland soils are Kaikoura, Ruahine, Makara, and Te Ranga steepland soils.

* Such cases have been observed.

Total porosities and macroporosities are high for the first two types of this group, but for Makara and Te Ranga steepland soils there is a decrease in these measurements. There appears to be no trend in the results for available moisture.

The soils on rolling slopes and under conditions of moderate weathering are, in order of increasing weathering: Owaka, Belmont, and Korokoro silt loams, and Taita clay loam. It should be pointed out that Taita clay loam is derived from material of prior origin to that of the other soils in this group. However, for Owaka and Korokoro silt loams, and Taita clay loam, the topsoil total porosities and macroporosities are similar; in the subsoil, results for both these measurements are low, with total porosity decreasing slightly with weathering. Both in topsoil and subsoil, figures for aggregate stability decrease with weathering for these three soils, notably so for Taita clay loam. Belmont silt loam subsoil is exceptional among this group for its comparatively high total porosity and macroporosity. Available moisture and degree of aggregation figures are high in both horizons. The mechanical analysis figures indicate that, for some sites of this type, the subsoil has less clay than is usual in the group.

The strongly weathered soils on rolling stopes—Marua clay loam and Rangiora clay—are notable for their high clay content. Probably due to this factor figures for aggregate stability are also high. Although topsoil available moisture is not low (indeed, for Marua clay loam it is fairly high), the low figures for available moisture in the subsoil, coupled with the shallow depth of topsoil, indicate that for a given depth of soil these two types will not store as much available water as the lesser weathered soils. In both horizons total porosity figures are similar for the two types but macroporosities are lower in Rangiora clay.

Renata silt loam—a podzolised soil in the moderately weathering zone—may be compared to Rangiora clay. Topsoil total porosities are similar for both types but Renata silt loam has the higher values in the subsoil. Macroporosity and figures for aggregate stability are similar in both types. However, available moisture is greater in Renata silt loam subsoil.

A comparison of the two moderately weathered groups—that on the steepland (Kaikoura, Ruahine, and Makara steepland soils) and that on rolling slopes (Owaka and Korokoro silt loams and Taita clay loam)—emphasises the generally higher total porosities and macroporosities of the former group. The stoniness of the steepland group possibly contributes to this difference.

The strongly weathered soils, Te Ranga steepland soil and Marua clay loam (a soil on rolling slopes), may also be compared. The textures of both types are similar. There is little difference between the other measurements for the two types, although the higher macroporosities for Te Ranga steepland soil are worth noting. Because of the thinness of topsoil it is doubtful whether the difference in available moisture is of practical significance.

Field Capacity

A consideration of the ratio $\frac{\text{field capacity}}{\text{moisture content at pF 2.31}}$ for several types showed that, for a particular horizon, the ratio was approximately constant for all sites of a type. This was used as a basis to accept or reject field capacity samples. (Samples were unacceptable probably mainly due to: (a) localised weather conditions not known to the person taking the samples; (b) failure of rain to wet the soil sufficiently.) In many cases repeat field capacity samples were found satisfactory and it would appear that such selection of samples is justified.

Because of the practical difficulties involved in obtaining samples at the correct time, it is often desirable to estimate field capacity from laboratory measurements. The regression equations connecting field capacity and (a) moisture content at pF 2.31 and (b) macroporosity, were derived for the results from both topsoils and subsoils taken separately and together. The best regression estimate of field capacity was:

$$\begin{matrix}\text{field capacity} \\ (\%\text{ by wt})\end{matrix} = -3.32 + 0.97 \begin{matrix}(\%\text{ moisture content} \\ \text{by wt at pF 2.31}).\end{matrix}$$

This estimate was obtained from topsoil and subsoil results together for 44 sites, the standard error of the regression coefficient being 0.04.

Packard (1957) also presented a relation between these two measurements for pumice soils, and it is worth noting that the line given in his paper is very similar to that represented by the above equation.

Percolation Rate

Knowledge of rates of infiltration of water into soils is obviously desirable, but, due to the difficulty of transporting the large quantities of water and other equipment required to most of the sites, it was decided to examine rates of water percolation through 4 in. diameter cores. Preliminary work with a seemingly homogeneous pasture soil showed that: (1) with topsoil cores the number of samples required was excessive; and (2) in the field the steady state rate of percolation into 4 in. diameter tubes appeared to be governed by the less permeable subsoil, and, in this case, the variation was less than for the topsoil. In an endeavour to reduce variation and permit a comparison of soil types, it was decided to compare percolation rates through subsoil cores.

Results are included in Table 2. Although no confidence limits have been placed on the percolation rates, it will be noted that, in general, the mean percolation rate increases with the macroporosity, and such a trend gives some value to the results. Similar trends have been presented by overseas workers: for example, Peele 1949; Smith *et al.* 1944. It appears possible that macroporosity measurements may be useful in estimating relative infiltration rates in New Zealand soils; however, more information is necessary on the variation of: (1) the

core percolation measurements, both in subsoil and topsoil; and (2) the relation of such measurements to actual infiltration rates. The large differences in percolation rates, not necessarily associated with large differences in macroporosity, suggest that attention should also be given to the size distribution of the macropores.

Conclusions

It is possible to characterise physically the modal profiles of New Zealand soils on greywacke by such measurements, and within such limits the results are probably relevant to plant growth. Investigation of one site should be sufficient, since means and confidence limits were non-separable as between one site and four sites. Also, physical measurements as herein are unlikely to differentiate between virgin and pasture modal sites provided such sites are morphologically similar.*

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* This will normally be the case.

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EFFECT OF SULPHUR DEFICIENCY ON SULPHUR AND NITROGEN LEVELS IN PASTURES AND LUCERNE

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Summary

Clovers, grasses, and lucerne from various trials have been analysed for total sulphur and nitrogen contents.

As with nitrogen, sulphur concentrations are higher in leaflets than in petioles in legumes, and higher in leaves than in stem tissue in lucerne and red clover. Levels are higher in younger than in older tissues.

The critical sulphur concentration in white clover at grazing height (3–6 in.) is approximately 0.26%. A similar or slightly higher level is indicated for grasses at the young leafy stage.

The critical level in lucerne at the hay stage is approximately 0.20% S.

In both white clover and lucerne, total nitrogen concentrations increased with yield increases from sulphur nutrient up to about 95% of the maximum yield.

Sulphur responses in legumes appear to be due to the nearly simultaneous correction of both sulphur and nitrogen deficiences.

Introduction

Tennent (1927) and Tennent and Hume (1928) obtained superphosphate responses in 23 out of 31 pasture top-dressing trials in Otago, but responses to basic slag at the same rate (2½ cwt/acre) and equivalent phosphorus content in only 8 of these trials.

Doak (1929) reported significant sulphur responses in lucerne on a Nelson soil, and in the same year Tennent and Duff (1929) recorded nearly as good responses in lucerne from a phosphorus-free sulphur fertiliser ("White Island product No. 1" containing about 22% S and 8.9% SO_3) as from the same quantity of superphosphate.

As superphosphate was quite satisfactory, providing both phosphorus and sulphur, little attention was paid to these findings until post-war shortage of sulphur and the introduction of double superphosphate and other sulphur-free phosphorus fertilisers focused attention on the problem. Superphosphate responses on soils with high phosphate tests also demanded investigation.

Pasture top-dressing trials laid down by farm advisory officers throughout New Zealand since 1952 have demonstrated that many of the superphosphate responses tacitly attributed to phosphorus were actually due to the sulphur component. All stages, from sulphur deficiency only to phosphorus deficiency only, have been found.

Lobb (1953, 1954) and Lobb and Reynolds (1956) have described results of observational trials in North Otago. More detailed work in Canterbury has been described by Walker *et al.* (1955, 1956) and Walker and Adams (1958). In a review of South Island work, Lobb (1959) has pointed out that sulphur deficiency occurs on a wide range of soil-types, derived from very different parent materials, and under very different climatic conditions.

In the North Island, sulphur responses have been observed, particularly on newly developed pumice soils. The use of heavy dressings of superphosphate to correct phosphorus deficiency has tended to obscure the true sulphur status of many North Island soils.

The work described in this paper was commenced in May 1953, to see whether total sulphur concentrations in plant tissues could provide a useful guide to the sulphur status of pasture species and lucerne.

Experimental

Plant samples were taken from various trials conducted by the Farm Advisory Division, New Zealand Department of Agriculture. These trials included replicated mowing trials with return of clippings; observational top-dressing trials, mostly under sheep grazing; and a pot trial. In the lucerne trials all cuts were removed. Pasture samples were taken mainly at 3–6 in. height, while still at a young vegetative stage of growth, and most were dissected into clover and grass components and analysed for sulphur and other major elements. The lucerne samples were taken at the hay stage, that is, at the commencement of flowering.

The treatment comparisons were double superphosphate (no S) against normal superphosphate (containing about 10% S as calcium sulphate), or double superphosphate against double superphosphate plus gypsum or finely ground sulphur.

Experiments from which samples were taken for analysis for total sulphur concentrations were as follows:

(1) *Mowing Trials with Measured Yields*

(A) *Pasture Trials*

Experiment 1. Lismore Stony Silt Loam under Irrigation. This 48-plot trial at Winchmore has been described by Lammerink (1959). Treatments were 4 rates of P (0, $\frac{3}{8}$, $\frac{3}{4}$, $1\frac{1}{2}$ cwt mono-calcium phosphate/acre) by 4 rates of S (0, $\frac{1}{2}$, 1, 2 cwt gypsum/acre), applied 1 May 1957, 30 April 1958, and 3 July 1959.

Experiment 2. Marton Loam. This was a 64-plot multifactorial interaction trial carried out at Marton Experiment Station with a half replicate of all combinations of 7 nutrients at two rates. (See During *et al.* 1960). Half the plots received 64 lb sulphur/acre on 22 October 1952, and thereafter 20 lb/acre annually in the spring.

Experiment 3. Lismore Stony Silt Loam. This was a 64-plot interaction trial at Dromore (Ashburton); 56 lb S were applied on 9 August 1955, and 28 lb S in August 1956 and August 1957.

Experiment 4. Lismore Stony Silt Loam. This was a PS interaction trial at Winchmore Irrigation Research Station; gypsum applied at 144 lb/acre was compared with no sulphur, commencing March, 1958.

Experiment 5. Warepa Silt Loam. This was a 64-plot interaction trial at Invermay Research Station, Taieri; 56 lb S/acre were applied annually in autumn, commencing 23 February 1953. (See During *et al.* 1960).

Experiment 6. Kaingaroa Sand. This was a 64-plot interaction trial at Tauhara (Taupo); 30 lb S were applied 17 November 1955, and repeated 29 March 1957.

(B) *Lucerne Trials*

Experiment 7. Lismore Shallow Silt Loam. This was a 64-plot interaction trial at Winchmore; 56 lb S were applied on 30 November 1955, 28 lb in August 1956, 20 lb in August 1957, 40 lb in September 1958, and 40 lb in August 1959.

Experiment 8. Lismore Stony Silt Loam. This was an interaction trial at Dromore (Ashburton); 56 lb S were applied in August 1955, 28 lb in August 1956, and 28 lb in August 1957.

(2) *Pot Trial*

Tarawera Gravel. This was a white clover pot trial with 6 rates of gypsum (0, 14, 28, 56, 112, 224 lb/acre), conducted by E. B. Davies and F. D. Dorofaeff in 1955 with pumice soil from Te Teko.

(3) *Observational Trials, with Assessed Responses*

Samples were taken from sulphur trials on the following soil types:

South Island

(a) *Responses Recorded:* Kakahu, Katrine, Kowai, Lismore, Ngapara, Oamaru, Tekapo, Tekoa, Timaru, Waiareka complex.

(b) *No Responses Noted:* Otatara sands, Motukarara silt loam.

North Island

(a) *Responses Recorded:* Kaingaroa sand, Manawatu silt loam from pumice ash, Taupo sandy silt, Tarawera gravel, Waimarino sand.

(b) *No Responses Noted:* New Plymouth sandy loam, Hamilton clay loam, Hangaroa sandy loam, Horotiu sandy loam, Rukuhia peat.

Analytical

Steinbergs' digestion procedure (1953) has been used throughout. His turbidity method for the determination of sulphur in the extracts was first employed when the analytical work was commenced in 1953, but from 1956 the method of Johnson and Nishita (1952) and Johnson and Arkley (1954) was used. Difficulty was experienced with the latter method until all rubber connections between the nitrogen cylinder and the reaction flasks were replaced by plastic tubing, following discussions with Dr Johnson. After this change, reproducible results were obtained. Recoveries of sulphur were 97% from thiourea and 90% from cysteine and cystine but only 75% from methionine. Iismaa's work (1959) suggests that the poorer recoveries from methionine may be unimportant in pasture analyses. Better results have been obtained, in recent work, using nitric-perchloric acid digestion. The following figures indicate that the earlier results by the turbidity method, in the hands of the one operator who succeeded with the method, were comparable to those obtained by the more precise reduction method.

TABLE 1. Percentage S in Dry Matter

Method	Grasses				White Clover			
Turbidity method	.15	.17	.13	.16	.25	.23	.18	.22
Reduction method	.15	.14	.14	.16	.23	.19	.18	.24

With the Johnson method, the practice has been to run three reference standards, 3, 10, and 20μg S, with every new sample of reducing mixture (usually 800–900 ml, sufficient for three weeks). The coefficients of variation of the spectrophotometer readings for these three standards between April 1958 and September 1959 ranged from 1.3% to 1.7%.

Results

(1) *Relative Sulphur Levels in Different Tissues*

To determine the importance of stage of plant development and of specific tissue analysed, selected legume samples were separated into leaflet and petiole tissues in the case of white clover, and leaf and stem tissues in the case of red clover and lucerne. S and N levels were determined.

TABLE 2. Percentage S and N in Dry Matter of White Clover Leaflets and Petioles

Sample	Leaflet		Petiole		Whole Leaf	
	S %	N %	S %	N %	S %	N %
A (long petioles)	0.28	6.02	0.17	2.33	0.23	4.24
B (shorter petioles)	0.26	5.08	0.16	2.10	0.23	4.09

As with nitrogen, S levels are lower in the petiole than in the leaflet; consequently the proportion of petiole affects the S level in the whole leaf.

TABLE 3. Percentage S and N in Dry Matter of Lucerne and Red Clover

Type of Plant	Plant Height (in.)	Whole Leaves (Leaflets Plus Petioles)		Stems		Leaf Plus Stem	
		S %	N %	S %	N %	S %	N %
Lucerne							
Whole sample	24	0.36	4.47	0.16	1.51	0.22	2.18
Top 6 in.	24	0.38	4.60	0.23	2.17	0.33	3.90
Bottom 18 in.	24	0.35	4.01	0.11	1.26	0.19	1.85
Red Clover							
Whole sample	18	0.25	4.09	0.11	1.77	0.18	2.87
Top 6 in.	18	0.26	4.47	0.19	2.41	0.24	3.78
Bottom 12 in.	18	0.18	3.79	0.06	1.66	0.10	2.41

In legumes with an erect habit, such as red clover and especially lucerne, herbage samples include stem as well as leaf tissue. Results in Table 3 show that whole leaves (leaflets plus petioles) are much higher in S than stems. The lower S concentrations in stem tissues mean that S levels in red clover and lucerne herbage samples tend to be lower than in white clover. In 10 comparisons at a rather tall growth stage, the mean values for white clover and red clover growing in association were 0.215% and 0.141% respectively. The white clover samples consisted of whole leaves only, the red clover leaf plus stem tissues.

Younger tissues are also higher in sulphur than older, as exemplified in Table 3 by samples taken from the top 6 in. growth of lucerne and red clover at the hay stage, compared with the remainder of the sample. The ratio of stem to leaf tissue increases with maturity; hence stage of growth affects sulphur concentrations in herbage samples. All these variables have to be considered in the interpretation of plant S levels and in arriving at critical values.

(2) *Relative Levels in Grasses and Clovers in Mixed Herbage*

Table 4 shows that: (1) in pastures adequately supplied with sulphur, the trend is towards rather higher values in grasses (dominantly rye-grass) than in associated clovers (mostly white clover); but (2) at deficiency levels, values tend to be similar, or the reverse trend is found.

In the overall comparison of grasses and clovers, 59 pairs showed higher S in grasses than in clovers, 22 were the same in both, and 43 were lower in grasses than in clovers.

Of the 68 pairs with grass levels above 0.25% S, 46 pairs showed higher S in grasses, 10 were the same in both, and only 12 had

TABLE 4. Mean S Levels (%) in Grasses and Associated Clovers

Type of Plant	All Samples (124 Pairs)	Grass S % >0.25 (68 Pairs)	Grass S % <0.25 (56 Pairs)
Grasses	0.286	0.323	0.210
Clovers	0.258	0.278	0.215

lower S levels in grasses than in clovers. Of the 56 pairs with grass S levels of 0.25% or less, only 14 pairs (9 with white clover) showed higher S levels in grasses than in clovers, 11 were the same in both, and 31 were lower in grasses than in clovers.

Part of this effect may be due to changes in the proportion of higher-fertility-demanding grass species, such as rye-grass and cocksfoot, as a result of sulphur treatments. Soil type may also influence the relative abilities of clovers and grasses to obtain their sulphur needs. The greatest contrast in uptake has been noted in two trials on slightly saline silt loam soils. At the Ahuriri lagoon (Napier) (soil pH 8, soluble salts 0.07%–0.14%), the mean levels in grasses in one cut were 0.38% S compared with only 0.25% in white clover; at Motukarara (Canterbury) (soil pH 5.6, soluble salts 0.07%) the corresponding levels were 0.44% and 0.26% S respectively.

The relation is further complicated by stage of growth. When a pasture is allowed to grow rather tall, approaching silage height, there may be an appreciable decrease in sulphur levels associated with development of fibrous stem and seed-head tissue in grasses, but the effect in the squat-growing white clover will be relatively small. This is illustrated in Table 5 by spring samples taken from three trials on different soil types in the Oamaru district, the first set being at a tall growth stage with some seed-head development in grasses, and the second from short-grazed pasture taken a year later from the same plots in the same trials.

TABLE 5. Relative S Levels (%) in Grasses and White Clover as Influenced by Stage of Growth

		Tall Growth November 1953		Short Growth October 1954	
		Grasses	White Clover	Grasses	White Clover
Exp. A	No S	0.17	0.28	0.21	0.21
	28 lb S	0.22	0.29	0.30	0.23
Exp. B	No S	0.13	0.18	0.20	0.16
	1 cwt S	0.27	0.27	0.25	0.22
Exp. C	No S	0.23	0.23	0.30	0.24

(3) *Relation Between Treatments and Yield Responses*

Approximately 1,000 observational top-dressing trials with sulphur have been laid down by farm advisory officers over a wide

range of soil types since 1952. In the earlier trials outstanding responses were obtained on deficient soils from applications of 28 lb and 56 lb flowers of sulphur, or 1 cwt gypsum/acre. In more recent trials rates of sulphur, ranging from 7 lb to 112 lb/acre and gypsum ½ cwt to 2 cwt/acre, have been compared on many sulphur-deficient South Island soils. On the most deficient soils, such as the foot-hill tussock country, best results have been obtained with the highest rates. In general gypsum has given more immediate responses than elemental sulphur, especially when the latter is rather coarsely ground, but effects of gypsum have been less prolonged, especially in higher rainfall districts. Similar trends have been noted on the pumice-covered Wairoa-Gisborne hill country in the North Island. On dry soils gypsum appears to be more effective than finely ground sulphur.

(4) *Relation Between Treatments and Sulphur Levels in Plant Tissues*

Except where very small amounts of sulphur have been applied, sulphur treatments have nearly all been reflected in increased total S concentrations, whether on sulphur-deficient or sulphur-adequate soils. Where heavy dressings of superphosphate have been applied to give maximum yields on soils with high phosphate-fixing properties, total sulphur concentrations in excess of 0.6% have sometimes been found, more particularly in grasses.

(5) *Relation Between Yield Responses and Sulphur Levels in Plant Tissues*

The main objective of the present work has been to establish "critical" or "threshold" levels for sulphur in plant tissues. The concept of critical values has been discussed in a paper on potassium deficiency (McNaught 1958). Nutrient concentrations in plant tissues at about 90% of the apparent maximum yields were used in that work. As more precise estimates were possible in some of the sulphur trials, 95% of the apparent maximum yields have been used in the present work.

Pasture Mowing Trials

Experiment 1. Lismore Stony Silt Loam, Winchmore

The mean dry matter yields for the cuts analysed are given in Table 6; yields for spring 1957 and 1958, which were statistically analysed, have also been included.

Significant differences obtained by using Duncan's test (1955) are shown for the spring yields. In comparing any two values within a set the differences are significant when there are no letters in common. The small letters refer to the 5% level of significance, capital letters to the 1% level.

There were yield responses to both P and S and highly significant P S interactions on yield, but the interactions on plant S levels were slight. The main effects of rates of P on S levels in the cut of 7 October 1957 are shown in Table 7. The differences were not significant.

TABLE 6. DRY MATTER YIELDS PER ACRE (lb)

Date	Gypsum Per Acre			
	Nil	½ cwt	1 cwt	2 cwt
16 Sept. 1957	368	595	590	643
7 Oct. 1957	598	803	838	885
30 Oct. 1957	625	845	883	913
Spring 1957	1,850	2,580	2,680	2,830
	cC†	bB	bAB	aA
6 Oct. 1958	252	634	631	626
Spring 1958	2,150	3,010	3,070	3,080
	bB†	aA	aA	aA
6 Oct. 1959	356	588	601	608

† Meanings of letters are explained in text (page 183).

TABLE 7. MAIN EFFECTS OF P ON S LEVELS (%) IN WHITE CLOVER AND GRASSES

Type of Plant	Mono-Calcium Phosphate Per Acre			
	Nil	⅜ cwt	¾ cwt	1½ cwt
White clover	0.263	0.275	0.267	0.269
Grasses	0.268	0.275	0.270	0.267

It is generally assumed that some build-up of a nutrient can be expected if another limiting factor is operating to reduce responses. This is not confirmed for sulphur levels in this experiment, where phosphorus is a second limiting factor. The sulphur levels (Table 7) in phosphorus-deficient plants with relatively low yields were very similar to those in higher-yielding plants from the high phosphorus plots with the same S treatments. For this reason the percentage S values for different rates of P have been pooled in Table 8. The 1958 and 1959 analyses were restricted to the highest phosphorus rate.

TABLE 8. MEAN PERCENTAGE S IN THE DRY MATTER OF WHITE CLOVER AND GRASSES

Type of Plant	Date	Gypsum Per Acre			
		Nil	½ cwt	1 cwt	2 cwt
White clover	16 Sept. 1957	0.215			0.297
White clover	7 Oct. 1957	0.218	0.273	0.284	0.299
		dC	cB	bAB	aA
Grasses	7 Oct. 1957	0.220	0.268	0.278	0.313
		cC	bB	bB	aA
White clover	30 Oct. 1957	0.190	0.220	0.255	0.263
Grasses	30 Oct. 1957	0.185	0.190	0.248	0.248
White clover	6 Oct. 1958	0.217	0.260	0.270	0.297
White clover	5 Oct. 1959	0.207	0.250	0.270	0.327

Assuming the yields at the 2 cwt rate were at the maximum, a critical level of 0.25% to 0.28% S (mean 0.26%) is indicated from this trial for spring growth of both white clover and grasses under irrigation. The grasses were 50% perennial rye-grass, the balance mainly cocksfoot and timothy.

The effect of the sulphur treatments on total nitrogen levels is summarised in Table 9.

TABLE 9. Mean Percentage N in the Dry Matter of White Clover and Grasses

Type of Plant	Date	Gypsum Per Acre			
		Nil	½ cwt	1 cwt	2 cwt
White clover	16 Sept. 1957	4.92			5.19
White clover	7 Oct. 1957	5.04	5.42	5.52	5.56
		cB	bA	aA	aA
Grasses	7 Oct. 1957	3.59	3.77	3.74	3.79
		bB	aA	aA	aA
White clover	30 Oct. 1957	4.54	4.92	4.95	4.97
Grasses	30 Oct. 1957	2.73	2.91	2.95	2.89
White clover	6 Oct. 1958	4.55	4.97	5.13	5.36
White clover	5 Oct. 1959	4.74	5.72	5.88	5.67

There was a 50% increase in the percentage of clover from a mean value of 21% to a mean of 31% in the 1957 cuts, as a result of the application of ½ cwt gypsum/acre. Further slight increases to 34% and 36% respectively were obtained from the 1 cwt and 2 cwt rates. Increases in nitrogen concentrations in the white clover roughly paralleled these increases in the percentage of clover. Yields of mixed herbage (70%–80% grasses) increased in a similar pattern (Table 6). Nitrogen concentration in the grasses increased significantly with the ½ cwt rate.

Experiment 2. Marton Loam

In this trial there were no significant sulphur responses in the first year (November 1952 to November 1953); in the second year there was a 2% overall response (significant at the 5% level); in the third 5%; in the fourth 4%; and in the fifth year 11%; all were significent at the 1% level. The fact that there were highly significant responses to both lime and sulphur suggests that the latter responses were due to the correction of sulphur deficiency and not to pH changes.

Pasture samples were analysed from cuts of 18 December 1953 and 23 October 1957, the latter just before the trial was closed. Mean sulphur levels in the 1953 samples of mixed herbage from eight plots without S and eight with S were 0.40% and 0.44% respectively. There were no sulphur responses at this date or in earlier cuts.

In the cut of 23 October 1957, mean S and N levels in white clover and grasses (37% *Poa sp.*, 28% cocksfoot, 24% perennial ryegrass) were as shown in Table 10.

TABLE 10. Percentage S and N in Grasses and White Clover

Type of Plant	No S		Plus S	
	S %	N %	S %	N %
White clover	0.234	5.41	0.253	5.59
Grasses	0.262	3.47	0.307	3.46

Yield evidence from the previous season suggests that the annual rate of sulphur used (20 lb/acre) may have been barely sufficient to ensure maximum yields for the whole 12-months period. For these reasons it is considered likely that the mean sulphur level of 0.25% in the white clover from the plus S treatment in October 1957 is close to the critical level. In this trial, grass S levels are higher than in Experiment 1, suggesting a critical level of about 0.30%.

The sulphur treatment gave a highly significant overall increase in N concentration in the white clover (Table 10). Phosphorus had a similar effect in the plus S plots, while potassium depressed the levels. There were significant positive SP and KP interactions on N levels. The interaction table below (Table 11) shows that the increase in N levels resulting from sulphur applications was much greater with the high rate of phosphorus than with the low rate. The greatest increases were obtained in the presence of high rates of both phosphorus and potassium, that is, in the plants most deficient in sulphur, since there were yield responses to both P and K.

TABLE 11. SPK Interactions on Percentage N in White Clover

	Low K		High K	
	Low P	High P	Low P	High P
No S	5.56	5.49	5.25	5.36
Plus S	5.60	5.72	5.39	5.63
Differences	.04	.23	.14	.27

Experiment 3. Lismore Stony Silt Loam, Dromore

White clover dissected from the cut of 24 September 1957 was analysed for sulphur content. The mean of samples from plots without S was 0.21%, and with S 0.25%. The corresponding N levels were 4.72% and 5.15% respectively. The response at the time of

sampling was 16%. As the last treatment of 28 lb S was applied on 5 August 1957, it is likely that it had not shown its full effect on plant S levels by September. It is considered that the analytical levels are consistent with a critical level of 0.25% to 0.26% S for white clover.

Experiment 4. Lismore Stony Silt Loam, Winchmore

The mean sulphur level in samples of white clover from eight plots without S was 0.230% (range 0.20%-0.26%), and from eight plots receiving 144 lb gypsum/acre was 0.295% (range 0.28%-0.31%). The mean response in this cut of 16 February 1959 was 39%. There is no evidence from the trial whether the 144 lb rate was excessive or just adequate. The critical level will certainly be above 0.23% and is likely to be about 0.28%.

Experiment 5. Warepa Silt Loam

Mixed pasture samples were analysed from the cut of 7 December 1953. Overall responses were obtained to molybdenum, lime, and phosphorus in the 1953-4 season, but not to sulphur. However, there was a significant positive LS interaction on yields. Sulphur increased yields on the high lime plots but depressed yields on the low lime plots because of an adverse effect on molybdenum availability. The mean sulphur level in eight samples from plots without S was 0.23%, and with S 0.25%. Plant analyses showed a very low nitrogen status (mean 2.5%) for the mixed herbage (about 40% clovers), and phosphorus levels were below optimum even where phosphorus had been applied. If all the other factors limiting growth had been corrected, leaving only the sulphur comparisons, clear-cut responses would have been expected.

Experiment 6. Kaingaroa Sand

Mixed herbage samples, taken 2 May 1956, showed mean S levels of 0.26% in the plots without sulphur and 0.29% in the sulphur-treated plots. The herbage consisted of 50% white clover, 25% red clover, and 20% grasses (mainly cocksfoot) in this cut, which was the first to show definite sulphur responses (highly significant overall response of 33%). The nitrogen, phosphorus, and potassium status of the herbage was very high, and growth was at a young succulent stage following autumn dry weather. The results are consistent with a critical level in white clover of about 0.28%.

TABLE 12. Percentage S, N and P in Grasses and Clovers

Type of Plant	No S			Plus S		
	S %	N %	P %	S %	N %	P %
Clovers	0.146	3.93	0.301	0.223	4.46	0.339
Grasses	0.131	2.74	0.426	0.199	3.36	0.409

Pasture samples taken 19 December 1956 from 16 of the plots were dissected into clovers and grasses and analysed, with the results shown in Table 12.

All differences between no S and plus S were significant at the 1% level, except for phosphorus in grasses.

The overall yield response from sulphur was 18% at this harvest, but 58% in the preceding cut (November) and 57% in the following cut (March). Growth in the December cut was rather long but still succulent. Clovers were dominantly red clover at the commencement of flowering, and included considerable stem tissue. Grasses were mainly cocksfoot. The quantity of herbage in the November cut was too great (nearly 2,000 lb dry matter/acre) to return to the plots without risk of smothering the plants. This probably temporarily depleted sulphur supplies (from oxidation of sulphur element by micro-organisms) and may explain both the relatively small sulphur response and the relatively low sulphur levels in the plant tissues.

Sulphur increased the nitrogen concentration in the grasses by nearly 23% and in the clovers by 14%. The relatively large increases in the grasses could be due in part to release of nitrogen through decomposition of clover roots and nodules (see Butler *et al.* 1959), following the drastic defoliation in November.

Pot Trial

Tarawera Gravel

Relative yields of white clover, and percentages of sulphur and nitrogen are shown in Table 13.

TABLE 13. Relative Yields and Percentage S and N in White Clover

	Relative Yields			March		April		June	
	March	April	June	S %	N %	S %	N %	S %	N %
Gypsum Nil	100	100	100	0.18	2.69	0.14	3.14	0.15	3.49
14 lb	100	131	147	0.19		0.19	3.76	0.14	3.60
28 lb	103	137	203	0.21		0.22	3.87	0.20	3.94
56 lb	**111**	133	256	**0.22**	2.96	0.24	4.20	0.21	4.22
112 lb	107	**145**	**261**	0.22		**0.25**	4.30	**0.25**	4.30
224 lb	102	131	258	0.25		0.26	4.24	0.24	4.35
Sig. diffs									
5%	NS	NS	56	0.021		0.030		0.028	
1%	NS	NS	75	0.028		0.041		0.037	
C.V.%	15.4	16.8	21.0	7.5		10.6		11.1	

The sulphur analyses were carried out in 1955 by the turbidity method (Steinbergs 1953). Two samples gave values of 0.18% S and 0.22% S by this method, and 0.18% S and 0.24% S respectively by the reduction method. Yields were variable owing to

insect damage and high soil temperatures, and to inadequate phosphorus, and differences did not reach significance in the first two cuts. Two samples from the 56 lb gypsum rate, from the April cut, showed P levels of 0.20% and 0.25%, which are below optimum for white clover. If adequate phosphate had been used, larger and more consistent sulphur responses could have been expected, without any decrease in sulphur concentrations in the plant tissues. (See Experiment 1).

Maximum yields (in bold type in Table 13) in each cut were obtained at the 56 lb or 112 lb rates, and associated sulphur levels were 0.22% to 0.25% (possibly 0.24% to 0.27% by the reduction method of analysis). Nitrogen concentrations also increased to a maximum near the 112 lb rate. The results are therefore considered to be consistent with a critical level of about 0.25% S for white clover grown in pots in the glasshouse.

Observational Pasture Top-dressing Trials

In the preliminary work, the observational technique was extensively used by advisory officers to find out which soil types were markedly deficient in sulphur. Plant samples were taken from some of these trials and analysed to correlate with the response evidence. The results are summarised in Table 14, together with results from the mowing trials.

TABLE 14. Relation Between S Concentrations and Responses

Type of Plant	Sulphur-Responsive Soils			Non-Responsive Soils	
	No Added S	Insuffic. S	Adequate S	No Added S	Plus S
Mixed pasture	0.204 (23) (.10 to .27)	0.229 (14) (.19 to .25)	0.301 (21) (.26 to .39)	0.319 (40) (.23 to .48)	0.368 (60) (.25 to .67)
Grasses	0.194 (37) (.10 to .30)	0.244 (56) (.16 to .28)	0.316 (26) (.25 to .46)	0.367 (7) (.30 to .44)	0.459 (7) (.34 to .77)
White clover	0.183 (76) (.08 to .27)	0.249 (72) (.18 to .29)	0.292 (22) (.25 to .34)	0.278 (30) (.23 to .32)	0.296 (14) (.26 to .49)
Red clover	0.142 (11) (.11 to .17)	0.22		0.207 (6) (.18 to .25)	0.203 (6) (.18 to .25)
Lucerne	0.164 (71) (.11 to .22)	0.17	0.240 (76) (.19 to .32)	0.379 (7) (.29 to .42)	0.449 (10) (.37 to .52)

Mean S levels are shown. (Number of samples, and range of values, in that order, are given in brackets.)

As the observational technique is limited to responses greater than about 15% to 20%, it is likely that some of the samples from soils classed as "non-responsive" may have been slightly deficient in sulphur. Soils with a history of superphosphate top-dressing may be unresponsive because of residual fertiliser sulphur or

accumulation of organic sulphur. If sulphur is withheld from such soils, mild deficiency may develop fairly rapidly, as in Experiment 2.

White clover from 10 trials in Canterbury and North Otago showed a mean N concentration of 3.1% in the control or low S plants (mean S level 0.14%), and 3.6% in the plus S plots (mean S level 0.20%). Severely deficient clover plants, with S contents of 0.09% S to 0.12% S, from rolling tussock country in several districts in Canterbury, showed N levels between 2.1% and 2.7%. As normal N levels in healthy white clover leaves are above about 4.5%, this suggests that total N levels may provide some measure of the severity of sulphur deficiency.

Lucerne Mowing Trials

Experiment 7. Lismore Shallow Silt Loam, Winchmore

Samples analysed from four cuts yielded the results shown in Table 15.

TABLE 15. Yield Responses and Percentage S and N in Lucerne

Date	Percentage Response	No S		Plus S	
		S %	N %	S %	N %
Dec. 1956	9.5	0.180	3.65	0.273	3.93
Jan. 1958	9.5	0.149	2.88	0.237	3.08
Jan. 1959	18	0.153	3.04	0.250	3.29
Dec. 1959	22	0.153	3.35	0.240	3.69

Highly significant yield responses to sulphur were obtained at each cut. A critical S level between 0.18% and 0.24% is indicated.

N levels were significantly higher in the plus S plots. No differences could be detected in non-protein nitrogen by the method described by Anderson and Spencer (1950). Two earlier cuts, taken before any S responses were found, showed no significant differences in N levels (3.80% and 3.80% in February 1956 and 4.50% and 4.41% in April 1956, for the no S and plus S plots respectively). (Corresponding yields/acre were 1,990 lb and 2,010 lb in February, and 1,255 lb and 1,200 lb in April).

As all 64 samples from the January 1958 set were analysed for S and N contents, a more detailed examination of the results is possible. In this cut there were highly significant yield responses to high lime (9.5%) and high potassium (6.8%), as well as the sulphur effects (9.5%). The sulphur response in plots receiving a low rate of lime was 11%, but in the presence of high lime was only 8%. Potassium and phosphorus had no effect on sulphur levels, but high lime gave an increase both in the sulphur-treated and untreated plots.

The increase in S levels suggests that the lime responses may have been due in part to increased availability of sulphur

TABLE 16. Effect of High Lime on Percentage S and N in Lucerne

	S %		N %	
	Low L	High L	Low L	High L
No S	0.141	0.157	2.77	3.00
Plus S	0.231	0.243	2.93	3.24

(molybdenum gave no responses). This is also consistent with the yield response evidence which showed a smaller sulphur response in the heavily limed plots.

Both high lime and sulphur increased N levels.

Experiment 8. Lismore Shallow Stony Silt Loam, Dromore

Mean sulphur levels in samples taken in November 1957, from each of the 64 plots, were 0.180% for the plots without S and 0.229% for the plus S plots. The overall growth response, significant at the 5% level, was 5.6%. Molybdenum also gave a response (5.7%), but the SMo interaction on yields was not significant. A critical level of approximately 0.20% S is indicated.

The SMo interactions on S and N levels are given in Table 17.

TABLE 17. Mean Percentage S and N in Lucerne

	No Mo		Plus Mo	
	S %	N %	S %	N %
No S	0.183	3.78	0.177	3.72
Plus S	0.229	3.79	0.229	3.80

There was no effect of sulphur on N levels in the absence of molybdenum, but a possible small effect in the presence of molybdenum. The results indicate that nitrogen fixation is not significantly affected when sulphur deficiency is slight. This trend is comparable to that found in white clover in the 1957 cuts in Experiment 1, where sulphur applications in excess of $\frac{1}{2}$ cwt or 1 cwt/acre gave only small further increases in N concentrations in clover despite significant yield increases.

Discussion

(1) *Clover-Rhizobium Symbiosis*

Anderson and Spencer (1950) found that sulphur-deficient subterranean clover plants showed symptoms of nitrogen deficiency with low percentage total nitrogen and that the plants were

indistinguishable in these characters from clover plants deficient in molybdenum. However, sulphur-deficient clovers contained more non-protein nitrogen than sulphur-treated plants and responded little in colour or growth to applied nitrogen fertiliser, whereas molybdenum-deficient plants were relatively low in non-protein nitrogen and showed good responses to combined nitrogen. These workers concluded: "Results suggest that the restricted growth of clover at low sulphur levels was not due to ineffective symbiotic nitrogen fixation, but to a deficiency of sulphur in the host legume, and that this condition exerted some controlling influence on symbiotic nitrogen fixation". They also stated that "the sulphur-deficient clover plants were not deficient in nitrogen for protein formation or growth". This last statement may be misconstrued as implying that nitrogen levels have no diagnostic value in sulphur deficiency investigations. Certainly the higher non-protein nitrogen status of the sulphur-deficient clovers indicated that nitrogen was not the *immediate* factor limiting growth. However, it is difficult to credit that the sulphur-deficient clovers containing only 1.9% total nitrogen in Anderson and Spencer's trials were not also deficient in nitrogen, especially as molybdenum-deficient clovers containing the same concentration of total nitrogen and higher protein nitrogen were highly responsive to nitrogen. Furthermore, these workers' evidence of sulphur effects on nodulation suggests a nitrogen deficiency. They found that an increase in percentage nitrogen in the host plant, induced by molybdenum or combined nitrogen, resulted in a decrease in nodulation, whereas sulphur increased both the percentage nitrogen and the nodulation. If, as Anderson and Spencer state, nodulation reflects the nitrogen demand of the host legume, an increase in nodulation resulting from sulphur application must mean that sulphur-treated plants need more nitrogen, that is, that sulphur applications aggravate an existing nitrogen deficiency.

It appears that sulphur-deficient clovers and lucerne dependent on symbiotically-fixed nitrogen are, for all practical purposes, *simultaneously* deficient in both sulphur and nitrogen. When rhizobia requirements for fixation are substantially *greater* than the needs of the host legume for growth, as in the case of molybdenum, a deficiency of this element will result in reduced fixation, and the host plant, within certain limits, will suffer from uncomplicated nitrogen deficiency. Such plants will respond appreciably to applied nitrogen fertiliser. If the rhizobia requirements for fixation are substantially *less* than the requirements of the host legume for growth, as in the case of potassium, a deficiency of this element will not appreciably affect fixation, as measured by the percentage total N in the tissues (McNaught unpublished data), in spite of interference in the nitrogen metabolism of the host plant (Richards and Berner 1954). When the needs of rhizobia and host plant are substantially the same, however, we can expect N levels to increase simultaneously with increasing levels of the other limiting factor, over the

responsive range for the host plant, as found in the present work on sulphur. Responses of the host plant to nitrogen fertiliser, as shown by Anderson and Spencer (1950), cannot take place in the absence of sulphur because this is essential for the formation of protein and therefore for growth; but sulphur responses can occur in nodulated plants, in the absence of fertiliser nitrogen, as a result of rapid mobilisation of the inexhaustible reserves of atmospheric nitrogen through increased fixation. The nitrogen-fixing activity of the rhizobia is itself governed by the host's immediate needs for nitrogen, and as soon as the small surplus of non-protein nitrogen is converted to protein, following application of sulphur, the bacteria rapidly respond to the resulting shortage of nitrogen by increased nodulation and by accelerated fixation. Although in such an association sulphur supplies must exert a controlling influence on symbiotic fixation, a sulphur response means a nearly simultaneous nitrogen response.

(2) *Critical Sulphur Levels in Legumes*

The results of the two lucerne trials suggest a critical level of approximately 0.20% S at the hay stage (commencement of flowering). Alway (quoted by Doak 1929) concluded that yield responses were likely when the S concentration fell below 0.25%, but Haddock and Vandecavaye (1946) set a lower value—0.15%. Bear and Wallace (1950) proposed an ideal concentration of 0.20% S. Rendig's data (1956) imply a critical level of this order.

With a pure legume stand, such as lucerne grown for hay, sulphur concentrations in tissues can be directly related to yield responses to sulphur fertilisers. In a mixed pasture association, however, the relation between sulphur levels in either clovers or grasses and responses is more complex, because of competition between grasses and clovers for light, moisture, and nutrients (including sulphur) and release of combined nitrogen by clovers to associated grasses. Yields of dissected clovers may not be the most satisfactory criteria to determine the critical sulphur level in clovers, as the objective is not to determine the minimum sulphur level associated with the greatest dominance of clover in the pasture, but to find the value associated with maximum yield of mixed herbage. Clover yields may be satisfactory at some times of the year or for a limited period following application of sulphur nutrient when grass growth is weak through nitrogen deficiency. Experiments with pure clover stands are needed for more precise information on clover requirements, but the internal needs of such plants may differ from those in a competitive association with grasses. In the present work, yields of mixed herbage have been used for assessing the critical level in white clover. The level indicated for white clover in pastures at grazing height is 0.25% to 0.29%, with an overall mean value of about 0.26%.

(3) *Critical Sulphur Levels in Grasses*

The grass-clover association is not comparable to the mutually controlled and beneficial symbiosis between clover and rhizobia. The grass benefits from the clover, but adversely affects clover

growth through competition. Grasses in association with clovers depend on release of nitrogen by excretion, or decomposition of foliage, roots, and nodules of clovers, and return of nitrogen by the grazing animal, especially in the form of urine. Differences in growth cycles between clovers and grasses and variable soil nitrogen supplies, especially under grazing, must mean a much more variable nitrogen status of grasses than of clovers and, consequently, much more variable yield responses to sulphur. The presence of this additional and uncertain factor of nitrogen deficiency limiting growth of grasses makes it difficult to determine the critical sulphur levels of grasses in a grass-clover association. It is not surprising, therefore, that the grass sulphur levels associated with near maximum yields in the pasture trials discussed in this paper show greater variability than the critical levels in white clover. Genetic differences may also contribute to this variability.

In Experiment 1 the apparent critical sulphur level for grasses (perennial rye-grass, cocksfoot, and timothy) was approximately 0.28% in the cut of 7 October 1957 when the nitrogen status was 3.7%, but only 0.25% in the next cut when the nitrogen level fell to 2.9%. In Experiment 2, where the grasses were *Poa sp.*, cocksfoot, and perennial rye-grass, a critical level of about 0.30% S was indicated, in association with a nitrogen status of 3.5%. In Experiment 5 the apparent critical level for mixed herbage (60% grasses, mainly perennial rye-grass, and 40% clovers) was about 0.23%. The nitrogen status of the grasses must have been very low, as the concentration in the mixed herbage was only 2.5% N.

These results suggest that the optimum sulphur level in grasses depends on the nitrogen status of those grasses; it is about 0.28% S to 0.30% S when the nitrogen concentration is high (3.5% to 4%), about 0.25% at a nitrogen level of 2.5% to 3%, and about 0.23% at 2.0% N to 2.5% N. If the term "critical level" is restricted to plants adequately supplied with other nutrients, the higher range of values (0.28% S to 0.30% S) must be taken as the tentative critical level in high-producing grasses at a young stage of growth.

Trials with pure stands and artificial nitrogen are needed to establish more precise critical sulphur levels in grasses.

Conclusions

(1) The critical sulphur level in white clover at grazing height is approximately 0.26% (range 0.25% to 0.29%). A similar level is indicated for mixed grasses (perennial rye-grass, cocksfoot, and timothy) in one trial, but a higher value, approximately 0.30%, for a *Poa*, cocksfoot, rye-grass mixture in another trial. The optimum sulphur level in grasses appears to be influenced by their nitrogen status.

(2) The critical level in lucerne at the hay stage (commencement of flowering) is approximately 0.20% S.

(3) Sulphur-deficient legumes are relatively low in total nitrogen content. The nitrogen levels appear to be roughly propor-

tional to sulphur levels. In severely deficient white clover plants with total sulphur concentrations below about 0.13% the nitrogen level may fall to as low as 2%, compared with a normal value of 4.5% to 5%.

(4) Sulphur responses in legumes appear to be due to the nearly simultaneous correction of both sulphur and nitrogen deficiencies.

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EFFECTS OF MOISTURE CONTENT ON IRRADIATED SEEDS OF *Lupinus angustifolius* L.

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Summary

Seeds of *Lupinus angustifolius* L. were conditioned to five different moisture contents and irradiated at three different doses.

Germination was reduced in irradiated seeds only at the highest moisture content (24%). Rate of germination was retarded in the irradiated seeds of low moisture content (6% and 9%).

Growth was retarded in all irradiation treatments. The effect increased with higher dose. Within any irradiation dose it was least at 12% and 16% moisture.

Death rate increased with increasing dose in each moisture treatment. It decreased in each irradiation treatment with increase in moisture up to 16%.

Critical dose and LD50 can be raised by appropriate conditioning of the seeds.

Introduction

Differences have been observed in irradiation injury in different lots of treated seeds of *Lupinus angustifolius* L. similarly irradiated in different years. Survival rates of plants varied from 15% to 70%. Such erratic results decrease the efficiency of projects designed to produce beneficial mutations. Variations in moisture content of irradiated barley seeds have been shown to affect survival and growth (Caldecott 1955). In this experiment a study is made of the effect on survival and growth of variations in moisture content of lupin seeds at the time of irradiation.

Material and Methods

Seeds of the variety Borre were conditioned to five different moisture levels in closed jars over chemicals of known vapour density. Dry calcium chloride, and saturated solutions of calcium chloride, sodium dichromate, sodium acetate, and calcium sulphate were used. These gave seed moisture contents of approximately 6%, 9%, 12%, 16%, and 24%, respectively. All seeds were treated with thiram dust before conditioning to prevent development of moulds, particularly likely to occur on seeds of high moisture content.

Seeds of each moisture level were divided into four lots of 30 seeds and sealed in polythene packets to maintain moisture content during transport and irradiation. They were given the following irradiation treatments over a Co^{60} source:

No irradiation

20.0 kr. at a dose rate of 17.1 r/min.

49.9 kr. at a dose rate of 19.4 r/min.

86.0 kr. at a dose rate of 22.2 r/min.

The seeds were sown two days after irradiation in 5 in. pots, one seed per pot, and grown in a greenhouse in a randomised block trial of six replications of 20 treatments.

The date of emergence of the seedlings, and the day on which the cotyledons had separated to an angle of 45°, thus showing the plumule, were noted. The day on which half of the cotyledon, mostly still covered with the testa, became visible was taken as date of emergence. The period taken up by germination was calculated by adding to the number of days between sowing and emergence half the number of days between emergence and separation of the cotyledons. This total will be referred to as the germination period.

Plant height was measured eight times, at weekly intervals, as the distance in cm from soil level to the stretched top of the highest leaves of plants.

After the eighth measurement plants were cut at soil level and their dry weight determined.

Results

Germination

The number of seeds of each treatment which germinated within 14 days, with the number that germinated later denoted in brackets, are shown in Table 1.

TABLE 1. Number of Seeds Germinated in 14 Days
(Later germinations are denoted in brackets)

	Moisture				
	6%	9%	12%	16%	24%
Dose (kr.)	Number of Seeds Germinated				
0	10 (17)	27	30	30	27
20.0	5 (25)	27 (3)	30	30	17
49.9	5 (25)	25 (3)	30	30	20
86.0	7 (23)	25 (2)	30	29	17

In the seeds conditioned to the 24% moisture level, germination of the irradiated seeds was significantly lower than that of the non-irradiated ($\chi^2 = 3.0$, $P < 5\%$). The seeds which did not germinate showed normal swelling, each with its testa intact; but the seed contents had rotted, although no moulds were visible.

There were no significant differences between any of the other treatments in the total number of germinated seeds.

No germination period shorter than seven days was recorded in any of the treatments. In many of the seeds conditioned to the 6% moisture, and a few seeds in the 9% treatment, germination was retarded.

No germination period longer than 14 days was recorded in the 12%, 16%, and 24% moisture treatments. Table 2 shows the distribution of germination periods at these moisture levels.

TABLE 2. Distribution of Germination Periods

	Moisture											
	12%				16%				24%			
Dose in kr.	0	20.0	49.9	86.0	0	20.0	49.9	86.0	0	20.0	49.9	86.0
Periods in days	Number of Seeds Germinated											
8	5	2	3	1	13	24	6	2	1	1	1	0
9	13	18	20	21	14	5	21	22	13	9	11	5
10	2	7	5	6	3	1	2	5	8	2	5	4
11 to 14	10	3	2	2			1		5	5	3	8
Total	30	30	30	30	30	30	30	29	27	17	20	17

The 20.0kr. 16% treatment germinated significantly earlier than the other 16% treatments. From 30 seeds 24 had germinated within 8 days ($\chi^2_{6\ d.f.} = 39.46$, $P < 0.1$). The number of seeds germinating within nine days was higher in all 16% moisture treatments than in any of the 12% and 24% treatments.

Growth

The mean heights of plants, in the various treatments, measured at weekly intervals, are shown in Table 3.

Plants from irradiated seeds grew less than the controls, except in the 20.0 kr. 16% treatment. Higher doses resulted in lower mean height. This effect was less marked at the 12% and 16% moisture level, and distinctly marked at certain stages of the growing period in the 49.9 kr., 9% and 24%, and 86.0 kr., 6%, 9%, and 24% treatments. In some treatments (49.9 kr. 24%, and 86.0 kr. 9%, 12%, and 24%) the mean height was smaller at a later date of measurement because the leaflets of the highest leaves dropped off after shrivelling; this decrease in height was

TABLE 3. Mean Heights of Irradiated Plants

Dose in kr.	Moisture in %	Measurements (cm) at Weekly Intervals							
		1	2	3	4	5	6	7	8
0	6	7.9	14.9	16.9	24.5	30.0	34.8	40.1	47.7
	9	7.2	13.6	17.4	24.0	31.3	38.5	46.2	55.7
	12	7.2	13.6	18.0	24.7	32.4	40.1	47.3	53.3
	16	8.4	14.2	18.3	25.0	32.1	40.0	47.2	56.0
	24	7.1	11.9	16.9	23.4	29.8	37.7	44.7	53.4
20.0	6	7.0	12.7	17.3	23.4	27.8	31.2	34.3	43.9
	9	5.1	11.2	14.9	20.5	27.8	35.0	42.1	49.3
	12	7.6	13.1	16.4	22.2	29.6	36.7	43.2	51.0
	16	9.0	14.5	18.3	24.8	32.4	39.9	46.5	54.0
	24	7.0	12.1	14.5	20.2	27.3	34.4	41.1	49.0
49.9	6	6.0	7.4	8.0	8.7	10.9	18.5	24.3	31.3
	9	4.0	5.7	6.8	8.1	11.4	16.3	19.5	27.0
	12	7.2	11.3	13.5	19.3	25.7	32.2	38.2	45.1
	16	6.9	10.8	12.7	17.6	23.7	29.6	35.9	42.9
	24	4.9	6.8	6.3	9.9	13.2	16.6	19.6	24.5
86.0	6	4.2	4.9	5.0	6.0	7.6	9.4	12.4	13.3
	9	3.7	4.1	4.4	4.1	4.2	4.6	5.1	5.8
	12	5.5	7.8	7.6	11.2	15.8	19.8	25.4	31.5
	16	6.4	9.6	10.2	14.6	19.3	25.7	31.3	38.9
	24	3.4	3.7	3.2	3.1	3.9	7.0	11.3	13.0

Dry weights of plants determined after the final measurement closely correlated with plant heights.

not noticed in the 6% moisture treatment, although shrivelling was apparent.

The growth rate of plants in all non-irradiated treatments was roughly linear over the growing period, with a slight drop at the second measurement. In all irradiated treatments the second interval was marked by a greater drop in growth rate, and this drop increased with higher doses. After the second interval the growth rate was as high as that in non-irradiated treatments for the treatments 20.0 kr. (all moistures), 49.9 kr. (12% and 16% moisture), and 86.0 kr. (12% and 16% moisture). Growth rates became normal for the 49.9 kr. 6% moisture by the fifth interval, and for the 9% moisture by the seventh interval; but they did not become normal in the 86.0 kr. 6%, 9%, and 24% moisture, nor in the 49.9 kr. 24% moisture, although growth was more nearly normal in the surviving plants after the fourth interval. Table 4 part A shows the interval in which full restoration of growth rate occurred.

Death

Table 4 part B shows the number of plants that died in each treatment, with the total number of germinated plants in brackets. Death rate increased with increasing irradiation dose in each

TABLE 4. Recovery, and Death, Following Irradiation

		Dose in kr.	Moisture				
			6%	9%	12%	16%	24%
A	Restoration Growth Rate. Interval	20.0	3	3	3	3	3
		49.9	5	7	3	3	—
		86.0	—	—	3	3	—
B	No. of Dead Plants	20.0	0 (27)	0 (30)	0 (30)	0 (30)	0 (17)
		49.9	20 (30)	10 (28)	0 (30)	0 (30)	1 (20)
		86.0	24 (30)	23 (27)	7 (30)	3 (29)	15 (17)
C	Occurrence of Death. Interval	20.0	—	—	—	—	—
		49.9	4	4	—	—	6
		86.0	3	4	4	6	6

moisture treatment and decreased in each irradiation treatment with increasing moisture up to 16%. The highest death rate occured in the 86.0 kr. 24% treatment.

Table 4 part C shows the interval between measurements of plant height in which the majority of deaths occurred.

Discussion

Klingmüller and Lane (1960) showed that conditioning seeds of *Vicia faba* L. to low moisture contents when not irradiated lowered germinative capacity and growth rate. They stressed the importance of this effect in the interpretation of the results of radio-biological work in dry seeds.

Conditioning of lupin seeds affected the rapidity of germination. Seed lots of low moisture levels, 6% and 9%, showed only partial germination after 14 days. Unswollen seeds were present which had not germinated. After scarifying, however, almost all of these seeds germinated within six days. Hyde (1954) has shown that drying may prevent subsequent entry of water into some normally soft legume seeds.

Conditioning of seeds has had no influence on plant growth, as is shown by the figures for plant height and weight.

Former experience with irradiated seeds of *Lupinus angustifolius* L. suggested that irradiation does not reduce germination capacity. This experiment confirms this finding, except for the 24% moisture content. From the figures it appears that irradiation under certain conditions can stimulate the capacity to germinate.

In this experiment the first symptoms of irradiation injury observed were mottling of the two first leaves and stunted growth

of seedlings. At first this mottling seemed to be equally severe for all irradiated treatments irrespective of dose and moisture content, but resulted in shrivelling of first leaves in certain treatments, as referred to before. New leaves do not show this mottling. Recovery of plants takes place with the development of new leaves, as does the restoration of growth rate.

Growth reduction has been used as a means of expressing the order of magnitude of irradiation injury. The severity of irradiation injury is affected by dose as well as by moisture content of the seeds at the time of irradiation. At a certain dose there is still difference in severity, due to difference of moisture content; a difference in severity still exists at a certain moisture level, due to difference in dose. The figures do not suggest a linear correlation between increase of dose and increase of injury, or decrease of moisture and increase of injury. Death occurs both above and below an optimum moisture level, but at different stages of growth—early in the case of low moisture, late in that of high.

These observations suggest a complexity of the nature of irradiation injury, consisting of at least two types of effects which could be described as:—

(1) Acute. The main effect is to cause physical damage to the embryo. Increase in susceptibility to the acute effect, it is suggested, could be brought about by decreasing the moisture of the seed.

(2) Chronic. These effects upset the metabolic system of seedling and plant. Increase in susceptibility to chronic effects could be produced, it is suggested, by increasing seed moisture.

Radio-sensitivity has been expressed as LD50, the lethal dose for 50% of the irradiated material, and Gustafsson (1944) has used the term "critical dose"—defined as the dose which leaves 37% of the material surviving to maturity. It has been shown in this experiment that appropriate conditioning of seeds of *Lupinus angustifolius* L. can raise the LD50 and hence the critical dose. It is probable that correct conditioning will result in a greater percentage of visible mutants from a given dose.

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